

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
12 September 2002 (12.09.2002)

PCT

(10) International Publication Number  
**WO 02/070436 A2**

- (51) International Patent Classification<sup>7</sup>: **C05F 11/08**
- (21) International Application Number: PCT/GB02/00906
- (22) International Filing Date: 1 March 2002 (01.03.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
- |            |                           |    |
|------------|---------------------------|----|
| 09/796,818 | 1 March 2001 (01.03.2001) | US |
| 09/796,819 | 1 March 2001 (01.03.2001) | US |
| 09/796,820 | 1 March 2001 (01.03.2001) | US |
| 09/796,821 | 1 March 2001 (01.03.2001) | US |
| 09/796,822 | 1 March 2001 (01.03.2001) | US |
- (74) Agents: MCCALLUM, William, Potter et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).



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## Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

**WO 02/070436 A2**

(54) Title: BIOLOGICAL FERTILIZER COMPOSITIONS COMPRISING MANURE, SLUDGE OR GARBAGE

(57) Abstract: The present invention provides biological fertilizer compositions that comprise yeast cells and an organic substrate. The yeast cells of the invention have an enhanced ability to fix atmospheric nitrogen, decompose phosphorus minerals and compounds, decompose potassium minerals and compounds, decomposes complex carbon compounds, overproduce growth factors, overproduce ATP, decompose undesirable chemicals, suppress growth of pathogenic microorganisms, or reduces undesirable odor. The organic substrates used can be manure, sludge, or garbage. The biological fertilizer composition of the invention can replace mineral fertilizers in supplying nitrogen, phosphorus, and potassium to crop plants. Methods of manufacture biological fertilizer compositions, and methods of uses are also encompassed.

**BIOLOGICAL FERTILIZER COMPOSITIONS  
COMPRISING MANURE, SLUDGE OR GARBAGE**

**1. FIELD OF THE INVENTION**

5       The invention relates to biological fertilizers that comprise yeasts and an organic substrate. The yeasts in the compositions of the invention have been stimulated to perform a variety of functions including the conversion of the organic materials into non-hazardous plant nutrients. The invention also relates to methods for manufacturing biological fertilizers, and methods for using the biological fertilizers to increase crop yields.

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**2. BACKGROUND OF THE INVENTION**

Use of fertilizer is essential in supporting the growth of high yield crops. Of the basic nutrients that plants need for healthy growth, large amounts of nitrogen (taken up as  $\text{NO}_3^-$  or  $\text{NH}_4^+$ ), phosphorus (taken up as  $\text{H}_2\text{PO}_4^-$ ), and potassium (taken up as  $\text{K}^+$ ) nutrients are required by most crops on most soils (Wichmann, W., et al., IFA World Fertilizer Use Manual). Such large amounts of nitrogen, phosphorus, and potassium nutrients are supplied mainly in the form of mineral fertilizers, either processed natural minerals or manufactured chemicals (K.F. Isherwood, 1998, Mineral Fertilizer Use and the Environment, United Nations Environmental Programme Technical Report No. 26).

15     Despite the importance of mineral fertilizers in providing mankind with abundant agricultural products, the harm done to the environment has been recognized in recent years. Mineral fertilizers may incurred damages to soils. For example, most nitrogen fertilizers may acidify soils, thereby adversely affecting the growth of plants and other soil organisms. Extensive use of chemical nitrogen fertilizers may also inhibit the activity of natural nitrogen fixing microorganisms, thereby decreasing the natural fertility of soils. The long term use of mineral fertilizers may also cause severe environmental pollution. For example, the loss of nitrogen and phosphate fertilizers due to leaching and soil erosion has led to contamination of soil and ground water, and eutrophication of surface water. Cleaning up polluted soil and water has been a complicated and difficult task. The cost for such a task is also astronomical.

20     In search for a solution to the problem, some are going back to organic fertilizers, such as manure (Wichmann, W., et al., IFA World Fertilizer Use Manual). The use of manure as fertilizer dates to the beginnings of agriculture. Large amounts of manure are produced by livestock. For example, in the United States, farms (including confined animal feeding operations) generate more than 136 million metric tons (dry weight basis) of

waste products annually. Manure has value in maintaining and improving soil because of the plant nutrients, humus, and organic substances contained in it. Studies have shown that a high percentage of the nitrogen, phosphorus, and potassium fed to dairy cattle are excreted in manure.

- 5        As manure must be managed carefully in order to derive the most benefit from it, some farmers may be unwilling to expend the necessary time and effort. Manure must be carefully stored to minimize loss of nutrients. It must be applied to the right kind of crop at the proper time. In general, manure does not provide all the plant nutrients needed and very large amount of organic fertilizers have to be applied to soil. Thus, there is  
10      a tendency to discount the value of manure as fertilizer. Manure may also contain undesirable chemicals, such as antibiotics and hormones. Only in underdeveloped countries, where artificial fertilizer may be costly or unavailable and where labor is relatively cheap, manure is attractive as a fertilizer.

- Furthermore, manure may contain significant levels of nitrogen and  
15      phosphorous which threaten water resources if not managed correctly. If not stored or disposed of properly, it can pose health and environmental threats. For example, it can cause air pollution, i.e., odor and dust; and contamination of surface and ground water with excess nutrients, organic matter, salts, and pathogens. For example, manure contains pathogenic microorganisms, such as *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, and  
20      *Campylobacter jejuni*.

- Biological fertilizers utilizing microorganisms have been proposed as alternatives to mineral fertilizers. Naturally occurring nitrogen fixing microorganisms including bacteria, such as *Rhizobium*, *Azotobacter*, and *Azospirillum*, (See for example, U. S. Patent No. 5,071,462) and fungi, such as *Aspergillus flavus-oryzae*, (See, for example, U. 25      S. Patent No. 4,670,037) have been utilized in biological fertilizers. Naturally occurring microorganisms capable of solubilizing phosphate rock ore or other insoluble phosphates into soluble phosphates have also been utilized in biological fertilizers either separately (e.g., U. S. Patent No. 5,912,398) or in combination with nitrogen fixing microorganisms (e.g., U. S. Patent No. 5,484,464). Genetically modified bacterial strains have also been  
30      developed and utilized in biological fertilizers. An approach based on recombinant DNA techniques has been developed to create more effective nitrogen fixing, phosphorus decomposing, and potassium decomposing bacterial strains for use in a biological fertilizer, see, for example, U.S. Patent No. 5,578,486; PCT publication WO 95/09814; Chinese patent publication: CN 1081662A; CN 1082016A; CN 1082017A; CN 1103060A; and CN  
35      1109595A.

However, the biological fertilizers that are based on naturally occurring microorganisms are generally not efficient enough to effectively replace mineral fertilizers. It is therefore important to develop more advanced biological fertilizers that can replace mineral fertilizers in supplying nitrogen, phosphorus, and potassium to crops for producing 5 high quality agricultural products while avoiding the problems associated with mineral fertilizers.

The present invention provides a biological fertilizer based on non-recombinant yeasts, which can replace mineral fertilizers and provide an effective and environmentally-friendly method of using certain organic materials.

10 Citation of documents herein is not intended as an admission that any of the documents cited herein is pertinent prior art, or an admission that the cited documents are considered material to the patentability of the claims of the present application. All statements as to the date or representations as to the contents of these documents are based on the information available to the applicant and does not constitute any admission as to the 15 correctness of the dates or contents of these documents.

### 3. SUMMARY OF THE INVENTION

The present invention relates to biological fertilizer compositions. The 20 biological fertilizer compositions of the invention comprises up to nine different yeast cell components, an organic substrate component, and optionally an inorganic substrate component. In particular, the yeast cell components of the composition are each capable of at least one of the following ten functions, namely, fixing atmospheric nitrogen, decomposing insoluble phosphorus or potassium minerals, maintaining a balance of 25 phosphorus compounds in the microenvironment, decomposing complex carbon-containing materials or compounds, overproducing growth factors, overproducing ATP, suppression of growth of pathogenic microorganisms, breakdown of undesirable chemicals, and reducing the odor of organic matters, respectively. The organic substrate component may include manure, sludge, or garbage. The yeast cell components of the invention can be used as an 30 additive which is mixed with an organic material to form a biological fertilizer.

In one embodiment, the biological fertilizer compositions of the invention are produced by mixing an organic substrate component with at least seven and up to nine yeast cell components, wherein the cells of six yeast cell components perform the basic functions of fixing atmospheric nitrogen, decomposing phosphorus-containing minerals or 35 maintaining in its immediate surroundings a balance of phosphorus compounds,

decomposing potassium-containing minerals, decomposing complex carbon-containing materials or compounds, overproducing growth factors, and overproducing ATP, and wherein the cells of the other component(s) perform the supplementary functions of suppressing growth of pathogenic microorganisms, decomposing undesirable chemicals,  
5 and reducing the odor of the organic substrate in the fertilizer composition.

In preferred embodiments, the present invention uses yeasts that are commercially available and/or accessible to the public, such as but not limited to *Saccharomyces cerevisiae*. Generally, the yeast cell components of the invention are produced by culturing the pluralities of yeast cells under activation conditions such that the  
10 abilities of the pluralities of cells to perform the functions are activated or enhanced. Accordingly, in another embodiment, the invention encompasses methods of activating or enhancing the abilities of yeast cells to perform one of the ten functions. The invention also relates to methods for manufacturing the fertilizer comprising mixing the organic substrate component with the yeast cells of the present invention, followed by drying and packing the  
15 final product.

The invention further relates to methods for using the fertilizer compositions of the present invention. The biological fertilizer compositions of the present invention are used to support and enhance the growth and maturation of a wide variety of plants.

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#### 4. BRIEF DESCRIPTION OF FIGURES

Fig. 1 Activation of yeast cells. 1 yeast cell culture; 2 container; 3 electromagnetic field source.

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Fig. 2. Formation of symbiosis-like relationships among strains of yeasts. 4 electromagnetic field source for nitrogen-fixing yeasts; 5 electromagnetic field source for P-decomposing yeasts; 6 electromagnetic field source for K-decomposing yeasts; 7 electromagnetic field source for C-decomposing yeasts; 8 yeast cell culture; 9 container.

30

Fig. 3. Adaptation of yeast cells to a soil type. 10 input electrode; 11 container; 12 electrode; 13 yeast cell culture; 14 electromagnetic field source; 15 temperature controller.

Fig. 4. Organic substrate grinding process. 16 organic raw material; 17  
35 crusher; 18 grinder; 19 organic substrate in powder form.

Fig. 5. Inorganic substrate grinding process. 20 inorganic raw material; 21 crusher; 22 grinder; 23 inorganic substrate in powder form.

5 Fig. 6. Yeast fermentation process. 24 activated yeast cells; 25 tank for  
culturing yeast cells, starch : water (35°C) = 1 : 2.5-3.5, semi-aerobic fermentation at 28 to  
30°C; 26 harvested culture.

10 Fig. 7. Mixing organic and inorganic raw materials. 27 inorganic materials;  
28 starch; 29 organic materials; 30 mixer; 31 mixture; 32 mixture to be transported to  
fertilizer production stage.

15 Fig. 8. Mixing yeast cells. 33 nitrogen-fixing yeasts 34 P-decomposing  
yeasts; 37 K-decomposing yeasts; 55 C-decomposing microbes; 35 ATP-producing yeasts;  
36 GF-producing yeasts; 52 pathogen-suppressing yeasts; 53 yeasts that decompose  
undesirable chemicals; 54 deodorizing yeasts; 38 mixture of yeasts; 56 mixture to be  
transported to fertilizer production stage.

20 Fig. 9. Fertilizer production process. 39 mixture of yeasts; 40 mixture of  
organic and inorganic materials; 41 granulizer; 42 fertilizer granules.

Fig. 10. Drying process. 43 fertilizer granules; 44 first dryer; 45 second  
dryer; 46 dried fertilizer.

25 Fig. 11. Cooling and packaging process. 47 dried fertilizer; 48 cooler; 49  
separator; 50 bulk bag filler; 51 final product.

## 5. DETAILED DESCRIPTION OF THE INVENTION

30 The present invention provides biological fertilizer compositions that  
comprise yeast cells and an organic substrate. The present invention also provides methods  
for manufacturing the biological fertilizer compositions as well as methods for using the  
biological fertilizer compositions.

The biological fertilizer compositions of the invention can replace  
35 chemical/mineral fertilizers in supplying nitrogen (N), phosphorus (P), and potassium (K) to

plants, especially crop plants. The inclusion of an organic substrate, such as manure, sludge, or manure in the biological fertilizer compositions of the invention provide an environmentally acceptable and economic method for recycling such materials.

- According to the invention, the biological fertilizer compositions comprise
- 5 poultry manure and a plurality of yeast cell components. Each yeast cell component is a population of yeast cells which comprises a plurality of yeast cells that are capable of performing a desired function. The yeast cell components of the invention can provide the following six basic functions: (1) fixation of atmospheric nitrogen; (2) decomposition of phosphorus minerals or compounds, or maintaining a balance of phosphorus compounds;
- 10 (3) decomposition of potassium minerals or compounds; (4) decomposition of complex or high molecular weight carbon materials or compounds; (5) overproduction of growth factors; and (6) overproduction of ATP. The yeast cell components of the invention can provide the following supplementary functions: (7) suppression of growth of pathogens, (8) degradation of undesirable chemicals, or (9) reducing the odor of organic materials.
- 15 In one embodiment, a biological fertilizer composition of the invention comprises (I) poultry manure; (II) at least one of the following yeast cell component: (a) a first yeast cell component comprising a first plurality of yeast cells that fix nitrogen; (b) a second yeast cell component comprising a second plurality of yeast cells that decompose phosphorus compounds; or (c) a third yeast cell component comprising a third plurality of
- 20 yeast cells that decompose potassium compounds; and (III) at least one of the following: (d) a fourth yeast cell component comprising a fourth plurality of yeast cells that suppress the growth of pathogenic microorganisms; (e) a fifth yeast cell component comprising a fifth plurality of yeast cells that degrade antibiotics; or (f) a sixth yeast cell component comprising a sixth plurality of yeast cells that reduce the odor of the biological fertilizer
- 25 composition. Thus, a biological fertilizer composition of the invention comprises at least two yeast cell components, one providing one of the three listed basic functions and one providing a supplementary function. In another embodiment, the biological fertilizer composition as described above further comprises at least one of the following: (g) a seventh yeast cell component comprising a seventh plurality of yeast cells that convert
- 30 complex carbon compounds to simple carbohydrates; (h) an eighth yeast cell component comprising an eighth plurality of yeast cells that overproduce growth factors; or (i) a ninth yeast cell component comprising a ninth plurality of yeast cells that overproduce adenosine triphosphate. In preferred embodiments, the biological fertilizer compositions of the invention comprises yeast cell components that provide all six basic functions, plus at least

one of the supplementary functions. Thus, the preferred biological fertilizer compositions comprise seven, eight or nine different yeast cell components.

The pluralities of the yeast cells of the invention can be added to any kind of manure, or existing organic fertilizers to improve their performance.

5       The organic substrates in the fertilizer compositions provides a source of nitrogen, phosphorus and potassium. Optionally, the fertilizer compositions may include an inorganic component comprising minerals which provides an additional source of phosphorous and/or potassium, and other minerals such as but not limited to calcium, magnesium, and sulfur; and micronutrients, such as but not limited to boron, copper, iron, 10 manganese, molybdenum, and zinc.

The biological fertilizer compositions of the present invention have many advantages over mineral fertilizers and organic fertilizers. Because the biological fertilizer of the present invention utilize metabolic activities of living yeasts to convert raw materials, such as atmospheric nitrogen, and phosphorus and potassium compounds in the substrate 15 component, into plant nutrients, the conversion and release of such nutrients by the yeast cells is regulated in part by the nutrient content of the soil. The nutrient content of the soil in turn depends in part on both the environment and the changing needs of plants. Therefore, the release of plant nutrients by the biological fertilizer compositions is adaptable to the soil condition and can be sustained over a period of time.

20      In addition to supplying nutrients to plants, the biological fertilizer compositions of the invention provide up to three supplementary functions that mitigate some of the undesirable properties of manure, sludge, or garbage that tend to restrict their use as organic fertilizers. The presence of pathogenic bacteria in manure, sludge, or garbage poses a health risk to humans and livestock. The biological fertilizer compositions can 25 include a component of yeast cells that can suppress the proliferation of pathogenic bacteria, thereby reducing the risk of infection, and circumventing the need to use chemicals in controlling the spread of such pathogens. Another yeast cell component that can be included in the composition is capable of reducing the odor of manure, sludge, or garbage , thus making its inclusion in a fertilizer more acceptable. Yet another yeast cell component 30 can be included to degrade undesirable chemicals, such as antibiotic feed additives, which are found in manure, sludge, or garbage. These supplementary functions generally lessen the adverse impact on the environment of using manure, sludge, or garbage in a fertilizer. The yeast cell components that provide the supplementary functions can each be separately included with the other six yeast cell components that provide the basic functions, or in

combination with each other and the other six components to provide the desired assortment of supplementary functions.

While the following terms are believed to have well-defined meanings in the art, the following are set forth to facilitate explanation of the invention.

5 As used herein, the term "nitrogen fixation" or "fixation of atmospheric nitrogen" encompasses biological processes in which molecular nitrogen or nitrogen in the atmosphere is converted into one or more nitrogenous (N) compounds, including but not limited to, ammonia, ammonium salts, urea, nitrites, and nitrates.

As used herein, the phrase "decomposition of phosphorus minerals or compounds" refers to biological processes which convert phosphorus (P) compounds, such as but not limited to those water-insoluble phosphorus compounds present in minerals, such as phosphate rock, into one or more different phosphorus compound(s) which are biologically available or more readily assimilable, i.e., usable for survival and/or growth, by plants and other yeasts. For example, the resulting phosphorus compounds may be more soluble in water or weak acid, and can thus be taken up by the roots of plants. Non-limiting examples of biologically available or assimilable phosphorus compounds include various classes of phosphates such as  $\text{PO}_4^{3-}$ ,  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ .

As used herein, the phrase "maintenance of a balance of phosphorus compounds" refers to biological processes which convert biologically unavailable or water-insoluble phosphorus compounds into one or more different phosphorus compound(s) which are more biologically available or soluble in water, wherein the processes are sensitive to excess or the lack of phosphorus (P) compounds in the local environment. The conversion process in the local environment is downregulated when the level of P compound is high (i.e., greater than about 180 ppm) and upregulated when level of P compound is low (i.e., greater than about 60 ppm)

As used herein, the phrase "decomposition of potassium minerals or compounds" refers to biological processes which convert potassium (K) compounds, such as but not limited to those water-insoluble potassium compounds present in potassium-containing minerals and materials, into one or more different potassium compound(s) which can be biologically available or more readily assimilable by plants and other yeasts. For example, the resulting potassium compounds may be more soluble in water, and can thus be taken up by the roots of plants.

As used herein, the phrase "decomposition of complex or high molecular weight carbon minerals, materials or compounds" refers to the biological conversion of a complex organic or inorganic carbon molecule (e.g. complex carbohydrates like cellulose

and lignin) into one or more carbon compound(s) which are of a lower molecular weight (e.g., simple carbohydrates) and which can be readily used for survival and/or growth by plants and yeasts. This process includes those reactions where long chains of carbon atoms in a polymeric carbon compound are cleaved.

- 5        As used herein, the term "growth factors" refers to molecules commonly required for the growth of yeasts, including but not limited to vitamins, in particular, vitamin B complexes, e.g., vitamin B-1, riboflavin (vitamin B-2), vitamin B-12, niacin (B-3), pyridoxine (B-6), pantothenic acid (B-5); folic acid; biotin; para-aminobenzoic acid; choline; and inositol.
- 10      For the purpose of this invention, the above-described five functions together with the overproduction of growth factors and ATP are referred to as the basic functions.
- As used herein, the phrase "suppressing the growth of pathogens" refers to a decrease or lack of increase in the number of pathogenic microorganisms present in a sample of manure, sludge, or garbage over a period of time, as a result of the presence of the yeast cells of the invention in the sample. It is to be understood that in the absence of the yeast cells, the number of pathogens in the sample would increase naturally. Many such microorganisms cause diseases in humans and animals, and may include bacteria such as *Escherichia* species, *Salmonella* species, *Shigella* species, *Mycobacterium* species, *Staphylococcus* species, *Bacillus* species, *Streptococcus* and *Diplococcus* species.
- 15      As used herein, the phrase "degradation of undesirable chemicals" refers to biological or biochemical processes which result in the conversion of chemical compounds that are undesirable in a fertilizer to an inactive form, such as the breakdown of such compounds into lower molecular weight compounds. Antibiotics are commonly present in organic materials and such compounds are not desired in a fertilizer because of the potential risk of ingestion by humans, for example, by eating vegetables grown using a fertilizer comprising contaminated organic material, and the possible spread of antibiotic resistance in the environment. Many antibiotics are added to animal feed to protect various farm animals, such as chicken, turkey, and swine, from bacterial and parasitic diseases, and to promote growth. A significant amount of antibiotic feed additive is excreted by the animals,
- 20      and thus accumulates in manure and sludge. Many kinds of antibiotics have been used in animal operations, such as but not limited to aminoglycosides, tetracyclines, beta-lactams, glycopeptides, and macrolides. Examples of antibiotics approved for use in farms in United States include but are not limited to, bacitracin methylene disalicylate, bacitracin zinc, bambermycins, oxytetracycline, chlortetracycline, penicillin, tylosin/sulfamethazine,
- 25      roxarsone, nitrasone, monensin, lasalocid, carbodox, tiamulin, hygromycin B, nystatin,

novobiocin, sulfadimethoxine, ormetoprim, lincomycin, fenbendazole, and virginiamycin. The presence and quantity of such antibiotics in a composition can be determined by any methods known in the art, for example, high performance liquid chromatography (HPLC).

As used herein, the phrase "reducing the odor of organic materials" refers to

- 5 a process which results in a lower concentration of one or more odorous compounds in manure, sludge, or garbage. Odorous compounds, such as but not limited to hydrogen sulfide, ammonia, indole, skatole (i.e. 3-methyl-1H-indole), p-cresol, and organic acids, are known to contribute to the malodorous quality of manure, sludge, or garbage. The concentration of such malodorous compounds in poultry manure or in a sample of air in
- 10 contact with the manure can be determined by any method well known in the art, including but not limited to gas chromatography. Odor is a perception of smell by an organism with olfactory organs. A reduction of the intensity of the odor associated with manure, sludge, or garbage can be determined subjectively. Various methods and techniques are known to measure the intensity of an odor. One subjective measurement of odor intensity is to
- 15 measure the dilution necessary so that the odor is imperceptible or doubtful to a human or animal test panel. Alternatively, a recognition threshold may also be used which is a higher concentration at which the character of the odor is recognized. Any methods and techniques for objectively or subjectively determine the intensity of an odor can be used to monitor the performance of the compositions and methods of the invention.

- 20 For the purpose of this invention, the suppression of growth of pathogens, degradation of undesirable chemicals, and reduction of odor of organic materials are referred to as the supplementary functions or activities.

- 25 The inventor discovered that, under various culture conditions, yeasts can be induced to exhibit seven different basic functions and three supplementary functions. The culture condition determines the activity which is activated or enhanced in the cultured yeasts. The specific culture conditions for each of the ten functions are described in details in sections 5.1 to 5.10 respectively.

- 30 According to the invention, a yeast cell component of the biological fertilizer composition is produced by culturing a plurality of yeast cells in an appropriate culture medium in the presence of an alternating electromagnetic field or multiple alternating electromagnetic fields in series over a period of time. The culturing process allows yeast spores to germinate, yeast cells to grow and divide, and can be performed as a batch process or a continuous process. As used herein, the terms "alternating electromagnetic field", "electromagnetic field" or "EM field" are synonymous. An electromagnetic field useful in
- 35 the invention can be generated by various means well known in the art. A schematic

illustration of exemplary setups are depicted respectively in Fig. 1. An electromagnetic field of a desired frequency and a desired field strength is generated by an electromagnetic wave source (3) which comprises one or more signal generators that are capable of generating electromagnetic waves, preferably sinusoidal waves, and preferably in the 5 frequency range of 30 MHz - 3000 MHz. Such signal generators are well known in the art. Signal generators capable of generating signal with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output signal, and thus the field strength.

The electromagnetic field can be applied to the culture by a variety of means 10 including placing the yeast cells in close proximity to a signal emitter connected to a source of electromagnetic waves. In one embodiment, the electromagnetic field is applied by signal emitters in the form of electrodes that are submerged in a culture of yeast cells (1). In a preferred embodiment, one of the electrodes is a metal plate, and the other electrode comprises a plurality of wires configured inside the container (2) so that the energy of the 15 electromagnetic field can be evenly distributed in the culture. The number of electrode wires used depends on both the volume of the culture and the diameter of the wire. For example, for a culture having a volume of 5000 ml; one electrode wire having a diameter of between 0.1 to 1.2 mm can be used for each 100 ml of culture; for a culture having a volume greater than 1000 l, one electrode wire having a diameter of between 3 to 30 mm 20 can be used for each 1000 l of culture.

In preferred embodiments, yeasts of the genera of *Saccharomyces*, *Schizosaccharomyces*, *Sporobolomyces*, *Torulopsis*, *Trichosporon*, *Wickerhamia*, *Ashbya*, *Blastomyces*, *Candida*, *Citeromyces*, *Crebrothecium*, *Cryptococcus*, *Debaryomyces*, *Endomycopsis*; *Geotrichum*, *Hansenula*, *Kloeckera*, *Lipomyces*, *Pichia*, *Rhodosporidium*, 25 and *Rhodotorula* can be used in the invention.

Non-limiting examples of yeast strains include *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, 30 AS2.173, AS2.174, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380, AS2.382, AS2.390, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, 35 AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576, AS2.593,

- AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190,  
 AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009,  
 IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1043, IFFI 1045,  
 IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 1060, IFFI 1063, IFFI 1202,  
 5 IFFI 1203, IFFI 1206, IFFI 1209, IFFI 1210, IFFI 1211, IFFI 1212, IFFI 1213, IFFI 1215,  
 IFFI 1220, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI 1251, IFFI 1270, IFFI 1277,  
 IFFI 1287, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1292, IFFI 1293, IFFI 1297, IFFI 1300,  
 IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI 1309, IFFI 1310, IFFI 1311, IFFI 1331,  
 IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI 1339, IFFI 1340, IFFI 1345, IFFI 1348,  
 10 IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1411, IFFI 1413; *Saccharomyces cerevisiae* Hansen  
 Var. *ellipsoideus* (Hansen) Dekker, ACCC2043, AS2.2, AS2.3, AS2.8, AS2.53, AS2.163,  
 AS2.168, AS2.483, AS2.541, AS2.559, AS2.606, AS2.607, AS2.611, AS2.612;  
*Saccharomyces chevalieri* Guilliermond, AS2.131, AS2.213; *Saccharomyces delbrueckii*,  
 AS2.285; *Saccharomyces delbrueckii* Lindner var. *mongolicus* Lodder et van Rij, AS2.209,  
 15 AS2.1157; *Saccharomyces exiguum* Hansen, AS2.349, AS2.1158; *Saccharomyces fermentati* (Saito) Lodder et van Rij, AS2.286, AS2.343; *Saccharomyces logos* van laer et  
 Denamur ex Jorgensen, AS2.156, AS2.327, AS2.335; *Saccharomyces mellis* Lodder et  
 Kreger Van Rij, AS2.195; *Saccharomyces microellipsoides* Osterwalder, AS2.699;  
*Saccharomyces oviformis* Osterwalder, AS2.100; *Saccharomyces rosei* (Guilliermond)  
 20 Lodder et kreger van Rij, AS2.287; *Saccharomyces rouxii* Boutroux, AS2.178, AS2.180,  
 AS2.370, AS2.371; *Saccharomyces sake* Yabe, ACCC2045; *Candida arborea*, AS2.566;  
*Candida Krusei* (Castellani) Berkhout, AS2.1045; *Candida lambica*(Lindner et Genoud)  
 van.Uden et Buckley, AS2.1182; *Candida lipolytica* (Harrison) Diddens et Lodder,  
 AS2.1207, AS2.1216, AS2.1220, AS2.1379, AS2.1398, AS2.1399, AS2.1400; *Candida*  
 25 *parapsilosis* (Ashford) Langeron et Talice, AS2.590; *Candida parapsilosis* (Ashford) et  
 Talice Var. *intermedia* Van Rij et Verona, AS2.491; *Candida pulcherriman* (Lindner)  
 Windisch, AS2.492; *Candida rugousa* (Anderson) Diddens et Loddeer, AS2.511,  
 AS2.1367, AS2.1369, AS2.1372, AS2.1373, AS2.1377, AS2.1378, AS2.1384; *Candida*  
*tropicalis* (Castellani) Berkout, ACCC2004, ACCC2005, ACCC2006, AS2.164, AS2.402,  
 30 AS2.564, AS2.565, AS2.567, AS2.568, AS2.617, AS2.1387; *Candida utilis* Henneberg  
 Lodder et Kreger Van Rij, AS2.120, AS2.281, AS2.1180; *Crebrothecium ashbyii*  
 (Guilliermond) Routein, AS2.481, AS2.482, AS2.1197; *Geotrichum candidum* Link,  
 ACCC2016, AS2.361, AS2.498, AS2.616, AS2.1035, AS2.1062, AS2.1080, AS2.1132,  
 AS2.1175, AS2.1183; *Hansenula anomala* (Hansen) H et P sydow, ACCC2018, AS2.294,  
 35 AS2.295, AS2.296, AS2.297, AS2.298, AS2.299, AS2.300, AS2.302, AS2.338, AS2.339,

- AS2.340, AS2.341, AS2.470, AS2.592, AS2.641, AS2.642, AS2.635, AS2.782, AS2.794; *Hansenula arabitolgens* Fang, AS2.887; *Hansenula jadinii* Wickerham, ACCC2019; *Hansenula saturnus* (Klocker) H et P sydow, ACCC2020; *Hansenula schneggii* (Weber) Dekker, AS2.304; *Hansenula subpelliculosa* Bedford, AS2.738, AS2.740, AS2.760,
- 5 AS2.761, AS2.770, AS2.783, AS2.790, AS2.798, AS2.866; *Kloeckera apiculata* (Reess emend. Klocker) Janke, ACCC2021, ACCC2022, ACCC2023, AS2.197, AS2.496, AS2.711, AS2.714; *Lipomyces starkeyi* Lodder et van Rij, ACCC2024, AS2.1390; *Pichia farinosa* (Lindner) Hansen, ACCC2025, ACCC2026, AS2.86, AS2.87, AS2.705, AS2.803; *Pichia membranaefaciens* Hansen, ACCC2027, AS2.89, AS2.661, AS2.1039;
- 10 *Rhodosporidium toruloides* Banno, ACCC2028; *Rhodotorula glutinis* (Fresenius) Harrison, ACCC2029, AS2.280, ACCC2030, AS2.102, AS2.107, AS2.278, AS2.499, AS2.694, AS2.703, AS2.704, AS2.1146; *Rhodotorula minuta* (Saito) Harrison, AS2.277; *Rhodotorula rubar* (Demme) Lodder, ACCC2031, AS2.21, AS2.22, AS2.103, AS2.105, AS2.108, AS2.140, AS2.166, AS2.167, AS2.272, AS2.279, AS2.282; *Saccharomyces carlsbergensis* Hansen, ACCC2032, ACCC2033, AS2.113, AS2.116, AS2.118, AS2.121, AS2.132, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377, AS2.417, AS2.420, AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605, AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Saccharomyces uvarum* Beijer, IFFI 1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces willianus* Saccardo,
- 15 20 AS2.5, AS2.7, AS2.119, AS2.152, AS2.293, AS2.381, AS2.392, AS2.434, AS2.614, AS2.1189; *Saccharomyces sp.*, AS2.311; *Saccharomyces ludwigii* Hansen, ACCC2044, AS2.243, AS2.508; *Saccharomyces sinenses* Yue, AS2.1395; *Schizosaccharomyces octosporus* Beijerinck, ACCC 2046, AS2.1148; *Schizosaccharomyces pombe* Linder, ACCC2047, ACCC2048, AS2.248, AS2.249, AS2.255, AS2.257, AS2.259, AS2.260,
- 25 AS2.274, AS2.994, AS2.1043, AS2.1149, AS2.1178, IFFI 1056; *Sporobolomyces roseus* Kluyver et van Niel, ACCC 2049, ACCC 2050, AS2.619, AS2.962, AS2.1036, ACCC2051, AS2.261, AS2.262; *Torulopsis candida* (Saito) Lodder, ACCC2052, AS2.270; *Torulopsis famta* (Harrison) Lodder et van Rij, ACCC2053, AS2.685; *Torulopsis globosa* (Olson et Hammer) Lodder et van Rij, ACCC2054, AS2.202; *Torulopsis inconspicua* Lodder et van Rij, AS2.75; *Trichosporon behrendii* Lodder et Kreger van Rij, ACCC2055, AS2.1193; *Trichosporon capitatum* Diddens et Lodder, ACCC2056, AS2.1385; *Trichosporon cutaneum*(de Beurm et al.)Ota, ACCC2057, AS2.25, AS2.570, AS2.571, AS2.1374; *Wickerhamia fluorescens* (Soneda) Soneda, ACCC2058, AS2.1388.
- Certain yeast species that can be activated or induced according to the  
35 present invention and are included in the present invention are known to be pathogenic to

human and/or other living organisms, for example, *Ashbya gossypii*; *Blastomyces dermatitidis*; *Candida albicans*; *Candida parakrusei*; *Candida tropicalis*; *Citeromyces matritensis*; *Crebrothecium ashbyii*; *Cryptococcus laurentii*; *Cryptococcua neoformans*; *Debaryomyces hansenii*; *Debaryomyces kloeckeri*; *Debaryomyces* sp.; *Endomycopsis fibuligera*. Under certain circumstances, it may be less preferable to use such pathogenic yeasts in the biological fertilizer of the invention, for example, if such use in an open field may endanger the health of human and/or other living organisms.

Yeasts of the *Saccharomyces* genus are generally preferred. Among strains of *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* Hansen is a preferred strain. The most preferred strains of yeast are *Saccharomyces cerevisiae* strains having accession numbers AS2.504, AS2.558, AS2.413, AS2.397, AS2.69, AS2.109, AS2.607, AS2.516, AS2.561, AS2.422, AS2.393, AS2.631, AS2.982, AS2.560, AS2.467, AS2.415, AS2.375, AS2.628, AS2.1190, AS2.562, AS2.463, AS2.409, AS2.379, AS2.666, AS2.631, AS2.182, AS2.431, AS2.606, AS2.53, AS2.611, AS2.414, AS2.576, AS2.483, IFFI 1211, IFFI 1293, IFFI 1308, IFFI 1210, IFFI 1213, IFFI 1307, IFFI 1206, IFFI 1052, IFFI 1301, IFFI 1291, IFFI 1202, IFFI 1021, IFFI 1059, IFFI 1052, IFFI 1441, IFFI 1008, IFFI 1220, IFFI 1302, and IFFI 1023 as deposited at the China General Microbiological Culture Collection Center (CGMCC).

Generally, yeast strains useful for the invention can be obtained from private or public laboratory cultures, or publically accessible culture deposits, such as the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 and the China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China.

The following yeast strains are preferred for making the P-balancing yeasts of the invention : AS2.558, AS2.118, AS2.103, AS2.132, AS2.121, AS2.189, AS2.216, AS2.265, AS2.417, AS2.420, AS2.200, AS2.162, AS2.440, AS2.277, AS2.441, AS2.443, AS2.444, AS2.605, AS2.595, AS2.638, AS2.742, AS2.748, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.109, AS2.112, AS2.375, AS2.560, AS2.561, AS2.562, AS2.559, AS2.501, AS2.502, AS2.503, AS2.504, IFFI1001, IFFI1002, IFFI1005, IFFI1006, IFFI1008, IFFI1009, IFFI1010, IFFI1012, IFFI1021, IFFI1027, IFFI1037, IFFI1042, IFFI1060, IFFI1063, IFFI1202, IFFI1203, IFFI1206, IFFI1209, IFFI1210, IFFI1211, IFFI1212, IFFI1213, IFFI1215, IFFI1220, IFFI1221, IFFI1224, IFFI1247, IFFI1248, IFFI1251, IFFI1270, IFFI1277, IFFI1287, IFFI1289, IFFI1290, IFFI1291, IFFI1292, IFFI1293, IFFI1297, IFFI1300, IFFI1301, IFFI1307, IFFI1308, IFFI1309,

IFFI1310, IFFI1311, IFFI1331, IFFI1335, IFFI1336, IFFI1337, IFFI1338, IFFI1340, IFFI1339, IFFI1345, IFFI1396, IFFI1399, IFFI1411, IFFI1413, IFFI1023, IFFI1032, IFFI1036, IFFI1044, and IFFI1207.

Although it is preferred, the preparation of the yeast cell components of the invention is not limited to starting with a pure strain of yeast. Each yeast cell component may be produced by culturing a mixture of yeast cells of different species or strains. The constituents of a yeast cell component can be determined by standard yeast identification techniques well known in the art.

Some yeasts may perform one of the desired functions more efficiently than others. The table below lists the species and accession numbers of various yeast strains and the preferred functions for which the respective strains are stimulated by the methods of the invention.

The ability and efficiency of any species or strain of yeast to perform any one of the ten desired functions before or after culturing under the conditions of the invention can readily be tested by methods known in the art. For example, the amount of nitrogen fixed can be determined by a modified acetylene reduction method as described in U.S. Patent No. 5,578,486 which is incorporated herein by reference in its entirety. The modified acetylene reduction method determines the amount of nitrogen fixed by measuring the decrease in molecular nitrogen in a volume of air. The amount of nitrogen fixed can also be determined by measurement of the ammonia and nitrates produced by the yeast cells (see, for example, Grewling et al., 1965, Cornell Agr Exp Sta Bull 960:22-25). A standard method that is applicable to determine total organic nitrogen is the Kjeldahl method.

The amount of phosphorus available to plants as a result of conversion from insoluble or biologically-unavailable phosphorus compounds can be determined by the molybdenum blue method (see, for example, Murphy et al., 1962, Analytica Chimica Acta 27:31-36) or the UV absorption method; whereas the amount of available potassium converted from insoluble or biologically-unavailable potassium compounds can be determined, for example, by flame atomic absorption spectroscopy (see, for example, Puchyr, et al., 1986, J. Assoc. Off. Anal. Chem. 69:868-870). The ability of the yeasts to supply biologically available N, P, and K after the biological fertilizer composition has been added to soil can be tested by many techniques known in the art. For example, plant-available ammonia, nitrates, P, and K produced by the yeast cells in soil can be extracted and quantitatively analyzed by the Morgan soil test system (see, for example, Lunt et al., 1950, Conn Agr Exp Sta Bull 541).

Methods well known in the art can be used for detecting and analyzing various organic molecules in manure and in soil, including HPLC. Similarly, methods well known in the art can be used for detecting and counting the number of viable microorganisms and the total number of microorganisms in a sample.

5 Without being bound by any theory or mechanism, the inventor believes that the culture conditions activate and/or enhance the expression of a gene or a set of genes in a yeast cell such that the cell becomes active or more efficient in performing certain metabolic activities which lead to the respective desired results.

As used herein, the term "organic substrate" refers to any kind of manure, 10 sludge, or garbage. In various embodiments, poultry manure, cattle manure, swine manure are used. Typically, the manure useful in the invention is produced by animals in animal operations such as but not limited to ranches, farms, slaughterhouses, and markets. The term "poultry manure" as used herein broadly encompasses organic material that comprises the feces and urine of domesticated birds, with or without accompanying litter such as straw, 15 hay, or bedding, that is traditionally used to fertilize land. Poultry manure includes but are not limited to manure produced by chicken, duck, turkey, goose, quail, squab, ostrich, and the like. Poultry manure include excrement or guano produced by non-domesticated bird species.

The term "cattle manure" as used herein broadly encompasses organic 20 material that typically comprises the feces and urine of domesticated ruminant mammals, such as dairy cows, beef cattle, with or without accompanying litter such as straw, hay, or bedding and that is traditionally used to fertilize land. The term "cattle manure" as used herein is not limited to just cattle but include other animals that graze, and that are kept primarily for their milk, meat, skin, hair, and pelts. Cattle manure includes but are not 25 limited to manure produced by buffalos, bisons, yaks, horses, donkeys, mules, sheep, goats, camels, and the like. Cattle manure also include excrement produced by non-domesticated herds.

The term "swine manure" as used herein broadly encompasses organic 30 material that typically comprises the feces and urine of swine, with or without accompanying litter such as straw, hay, or bedding and that is traditionally used to fertilize land. Swine manure includes but are not limited to manure produced by swines, hogs, pigs, and the like.

The term "sludge" as used herein broadly encompasses any solid matter that has settled out of suspension in the course of sewage storage and/or treatment, for example, 35 residues in a waste lagoon, in an urban sewage treatment plant. The term also include semi-

solid matters, and mixtures of effluent and sediments. The term thus encompasses sludge having a wide range of viscosity, density, and water content, as well as sludge which has been partially processed or stabilized.

- Solid waste, such as garbage, can also be used in the biological fertilizer compositions of the invention. Material that is discarded because it has served its purpose or is no longer useful is generally referred to as solid waste. The sources of solid waste include residential, commercial, institutional, and industrial activities. Non-industrial solid waste for example, those collected from urban areas, contains mostly discarded food materials or materials used in food preparation, and other dry materials, such as paper, fabric, or plastics. The type of solid waste most preferred for inclusion in the biological fertilizer compositions is garbage which has a high organic content (i.e., at least 30%). In residential and commercial areas (which have restaurants and hotels), garbage comprises mainly decomposable food wastes. Preferably, the solid waste used in the invention is separated from glass, metal, and other inorganic or non-decomposable items through sorting and separating operations. These can be carried out by the methods well known in the recycling / garbage disposal industry, such as mechanically, using differences in such physical characteristics of the solid waste as size, and density. Shredding or pulverizing reduces the size of the waste articles, resulting in a uniform mass of material which can be subjected to various processes, such as drying, etc.

- 20            Optionally, an inorganic substrate component can be included in the biological fertilizer compositions of the invention. The inorganic substrate component can include but not limited to phosphate rock or rock phosphate, apatite, phosphorite, sylvinitie, halite, carnalitite, and potassium mica.

- 25            Due to the variation of constituents in manure, sludge, or garbage, it may be desirable to subject a sample of a batch of the organic materials to analysis to determine the amount of plant nutrient present. Methods of soil analysis well known in the art can be used to measure the amount of N, P, K, calcium, magnesium, zinc, iron, manganese, copper, sodium and sulfur in the manure.

- 30            In various embodiments, the biological fertilizer compositions of the present invention each comprises at least seven yeast cell components capable of performing six basic functions plus at least one of the supplementary functions. In a most preferred embodiment, the biological fertilizer compositions comprise nine yeast cell components, in which case the six basic functions and all three supplementary functions are provided. It will be understood that alternative formulations are also contemplated.

In one particular embodiment of the invention, when a batch of the organic substrate that is relatively rich in biologically-available phosphorus is used, the biological fertilizer composition can be formulated to comprise yeast cells that can maintain a balance of phosphorus compounds instead of yeast cells that decompose phosphorus-containing minerals or compounds. Moreover, if desired, the biological fertilizer composition may comprise lesser quantities of one or more of the above-described yeast cell components that supply one of the six basic functions. For example, if the biological fertilizer composition is to be used in soil that is rich in potassium, the biological fertilizer composition can be formulated to comprise lesser amount of the yeast cells that can decompose potassium-containing minerals or compounds.

In another embodiment of the invention, where the yeast cells of the various yeast cell components are present in a mixture, the yeast cells can be cultured under certain conditions such that the yeast cells with different functions can supply each other with and/or rely on each other for nutrients and growth factors. As a result, a symbiosis-like relationship is established among the various yeast cell components in the fertilizer compositions of the invention. This culturing process is optional but can improve the stability and efficiency of the compositions such that the resulting fertilizer is made more suitable for long term use in natural soil environments. The culturing conditions for this optional process are described in Section 5.11.

In yet another embodiment of the invention, the yeast cells may also be cultured under certain conditions so as to adapt the yeast cells to a particular type of soil. This culturing process is optional, and can be applied to each yeast cell component separately or to a mixture of yeast cell components. The result is better growth and survival of the yeast cells in a particular soil environment. The culturing conditions for this optional process are described in Section 5.12.

As used herein, the biological fertilizer composition supports or enhances plant growth, if in the presence of the biological fertilizer in the soil, or applied to the roots, the plant gains viability, size, weight, rate of germination, rate of growth, or rate of maturation. Thus, the biological fertilizer compositions have utility in any kind of agricultural, horticultural, and forestry practices. The biological fertilizer compositions can be used for large scale commercial farming, in open fields or in greenhouse, or even in interiors for decorative plants. Preferably, the biological fertilizer is used to enhance the growth of crop plants, such as but not limited to cereal crops, vegetable crops, fruit crops, flower crops, and grass crops. For example, the biological fertilizer compositions may be

used with wheat, barley, corn, soybean, rice, oat, potato, apple, orange, tomato, melon, cherry, lemon, lettuce, carrot, sugar cane, tobacco, cotton, etc.

- The biological fertilizer compositions of the invention may be applied in the same manner as conventional fertilizers. As known to those skilled in the relevant art,
- 5 many methods and appliances may be used. In one embodiment, a mixture of culture broths of the yeast strains of the present invention and poultry manure are applied directly to soil or the roots of plants. In another embodiment, dried powders of the yeast strains of the present invention and poultry manure are applied to soil or the roots of plants. The biological fertilizer compositions may be applied to soil, by spreaders, sprayers, and other mechanized
- 10 means which may be automated. The biological fertilizer compositions may be applied directly to plants, for example, by soaking seeds and/or roots. Such application may be made periodically, such as once per year, or per growing season, or more frequently as desired. Although not necessary in most cases, the biological fertilizer compositions of the invention can also be used in conjunction or in rotation with other types of fertilizers.
- 15 In one preferred embodiment, the biological fertilizer composition of the invention, i.e., yeasts of the invention mixed with manure, dried sludge, or garbage in granular form, is used as a basal fertilizer which is applied into the soil at the depth of the major root system of the crop. Prior to application, the ground should be loosened and clear of weeds. The biological fertilizer composition can be spread evenly onto the ground,
- 20 added to holes or long furrows in the ground in the vicinity of the corona of a tree. For existing fruit trees, holes of about 5 to 30 cm deep is dug into which the biological fertilizer composition of the invention is added. The ground, holes, or furrows containing the biological fertilizer composition can then be covered with soil and watered thoroughly. After 3 to 7 days, the area is ready for planting or sowing. For rice, the ground is flooded with
- 25 water for 3 to 7 days before planting the seedlings. If used in sandy soil with a shallow root system, a depth of 5 to 15 cm is used; with a deep root system, 5 to 25 cm is recommended. In clay soil with a shallow root system, a depth of 2 to 10 cm is used; with a deep root system, 2 to 15 cm is recommended. The desired effect is that the biological fertilizer composition is contact with or in very close proximity to the roots of the plants. Preferably,
- 30 after application of the fertilizer and/or planting, the soil is not disturbed. Generally, the operation temperature of the fertilizer is between 5°C to 45°C, optimally between 16°C to 30°C; the preferred pH range is between 5.5 to 8.5, and optimally between 6.5 to 7.5.

Recommended Dosage

35	Crop	Amount of Biological Fertilizer
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	Vegetables (short-growing)	600 - 900 kg/ha
	Vegetable (long-growing)	900 - 1200 kg/ha
	Ground vegetable	900 - 1350 kg/ha
5	Solanaceous fruit	900 - 1350 kg/ha
	Root & Tuber vegetable	750 - 900 kg/ha
	Bulb vegetable	900 - 1200 kg/ha
	Legume	600 - 1050 kg/ha
10	Fruit Trees	2 - 5 kg/tree
	Paddy Rice	600 - 900 kg/ha
	Wheat & Corn	750 - 1200 kg/ha
	Cotton & Peanut	600 - 1200 kg/ha

15           Described respectively in Sections 5.1 - 5.10 are the yeast cell components used for nitrogen fixation, phosphorus compound decomposition, potassium compound decomposition, complex carbon compound decomposition, growth factors production, ATP production, pathogen suppression, degradation of undesirable chemicals, and reduction of odor. Methods for preparing each yeast cell components are described. Section 5.11  
 20          describes the methods for establishing a symbiosis-like relationship among yeast strains in a fertilizer composition of the invention. Section 5.12 describes methods for adapting yeast cells of the invention to a particular type of soil. Section 5.13 describes the manufacture of the biological fertilizer compositions of the invention. Methods for the preparation of organic substrates and for the manufacture of the biological fertilizer, including mixing,  
 25          drying, cooling, and packing, are also described. In various embodiments of the invention, standard techniques for handling, transferring, and storing yeasts are used. Although it is not necessary, sterile conditions or clean environments are desirable when carrying out the processes of the invention.

30           **5.1. NITROGEN-FIXING YEAST CELL COMPONENT**  
 Nitrogen fixation is a process whereby atmospheric nitrogen is converted into ammonia and nitrates. Close to 800 species of naturally occurring microorganisms, mostly bacteria and cyanobacteria, from more than 70 genera have been found to be able to fix nitrogen. Some of the nitrogen-fixing microorganisms, such as *Rhizobium*, form  
 35

symbiotic association with plants, especially in the root of legumes. Others, such as *Azotobacter*, are free-living and capable of fixing nitrogen in soil.

In the present invention, the ability of a yeast to fix nitrogen is activated or enhanced, and the resulting nitrogen-fixing yeast cells can be used as a component of the  
5 biological fertilizer compositions of the invention.

According to the invention, yeast cells that have an enhanced ability to fix nitrogen are prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing nitrogen fixation in yeasts can generally be found within the range of 800 MHz -  
10 1000 MHz. After the yeast cells have been cultured for a sufficient period of time, the cells can be tested for their ability to fix nitrogen by methods well known in the art.

The method of the invention for making the nitrogen-fixing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose,  
15 fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5%  
20 and 2%, and most preferably about 1%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, potassium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $\text{CaCO}_3$ ,  
25  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

Table 1: Composition for a culture medium for nitrogen-fixing yeast

Medium Composition	Quantity
$\text{KH}_2\text{PO}_4$	0.2g
$\text{K}_2\text{HPO}_4$	0.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.25g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	3.5g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.5g
NaCl	0.25g

Yeast extract paste	0.3g
Sucrose	12.0g
Distilled water or autoclaved water	1000ml

5

It should be noted that the composition of the media provided in Table 1 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

10

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of  $10^2$ - $10^5$  cell/ml, preferably  $3 \times 10^2$ - $10^4$  cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

15

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of about 800 to about 1000 MHz, preferably in the range of 840.000 to 916.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895, 900, 905, 910, 915, or 920 MHz. The field strength of the EM field(s) is in the range of 10 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

20

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., a few days to one or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 140 - 280 hours.

25

For example, using an exemplary apparatus as depicted in Figure 1, an initial EM field in the range of 10-20mV/cm, usually at about 12.5mV/cm is used. After this first

period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the EM field strength is increased to a higher level in the range of 50-200 mV/cm, usually to about 125 mV/cm. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to 5 conduct the process at 25° to 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the nitrogen-fixing yeast cells may be 10 recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The nitrogen-fixing yeast cells may also be dried and stored in powder form.

Any methods known in the art can be used to test the activated yeast cells for their ability to fix nitrogen. For example, a modified acetylene reduction method for 15 measuring nitrogen fixed by microorganisms is used to evaluate the nitrogen-fixing capability of the prepared yeast. The modified acetylene reduction method is described in U.S. Patent No. 5,578,486 which is incorporated herein by reference in its entirety. An alternative method based on 15-N can also be used.

The ability of the yeasts of the invention in fixing nitrogen can be 20 demonstrated by the following two methods:

One ml of activated yeast strain AS2.628 ( $2 - 5 \times 10^7$ ) was cultured in 1000 ml of Ashby medium at 28°C in the presence of a series of 8 EM fields in the order stated: 855 MHz at 14 mV/cm for 5 hours; 865 MHz at 14 mV/cm for 5 hours; 875 MHz at 14 mV/cm for 5 hours; 885 MHz at 14mV/cm for 5 hours; 855 MHz at 120 mV/cm for 30 25 hours; 865 MHz at 120 mV/cm for 30 hours; 875 MHz at 120 mV/cm for 30 hours; 885 MHz at 120mV/cm for 30 hours. In a separate container, as control, 1 ml of non-activated yeast was cultured under the same conditions without the EM fields. After culturing, the 1000 ml of yeast cells are mixed with 3000 g sterilized coal dust powder, and then dried at less than 70°C until the moisture content is less than 5%. The end product in powder form 30 (0.1 g) was sealed with 10 ml of Ashby medium in a 100 ml culture flask (5 flasks for each were used in the experiment). 10 ml of air was removed from the flasks by a syringe and replaced with 10 ml of acetylene (> 99% purity). The culture flasks were incubated at 28°C for 24-120 hours and the amount of acetylene reduced was measured by gas chromatography. The amount of acetylene reduced after 120 hours was greater than 120

µmol/g of the dried powder. There was no significant reduction of acetylene in the control containing non-activated yeasts.

Alternatively, the isotopic nitrogen dilution method can be used. The end product in powder form (0.1 g of non-activated and 0.1 g of activated yeasts) were cultured 5 separately for 96 hours at 28°C. The amount of nitrogen fixed by each was determined and compared. The amount of nitrogen fixed by activated yeasts was greater than 3.5 mg/g of the dried powder. The control containing non-activated yeasts did not show any significant fixation of nitrogen.

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## 5.2. PHOSPHORUS-DECOMPOSING YEAST CELL COMPONENT

The phosphorus compound-decomposing (P-decomposing) yeast of the invention converts insoluble or biologically-unavailable phosphorus-containing substances, such as phosphate rock, into soluble phosphorous compounds so that they become available 15 to plants.

In the present invention, the ability of yeasts to decompose insoluble phosphorus-containing substances is activated or enhanced, and the resulting P-decomposing yeast cells can be used as a component of the biological fertilizer compositions of the invention.

20 In various embodiments, P-decomposing yeast cells are employed in the compositions of the invention when the level of soluble or biologically-available phosphorous is low in the organic substrate. P-decomposing yeast is less preferred when the biologically-available phosphorous level is high which is common in poultry manure.

According to the invention, yeast cells that are capable of P-decomposing are 25 prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing P-decomposition in microbes can generally be found in the range of 300 MHz to 500 MHz. After the cells have been cultured for a sufficient period of time, the cells can be tested for their ability to decompose phosphorus-containing substances by methods well known in the 30 art.

The method of the invention for making the P-decomposing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in 35 combination as sources of assimilable carbon in the culture medium. The exact quantity of

the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 1.5%. These carbon sources can be used individually, or 5 several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, potassium, calcium, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ . Non-biologically available forms of phosphorus-containing substances in a 10 suitable form are also included in the media as dried organic substrate. Non-limiting examples of dried organic substrate include manure, sludge and garbage of  $\geq 150$  mesh. Other insoluble phosphorus-containing substances can also be used either separately or in combination.

15 Table 2: Composition for a culture medium for P-decomposing yeast

Medium Composition	Quantity
Sucrose	15g
NaCl	1.2g
20 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	3.0g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.3g
KNO <sub>3</sub>	0.3g
25 Yeast extract paste	0.5g
Dried manure, sludge, or garbage	1.2g to 2.4g; Powder of $> 150$ mesh
Autoclaved water	1000ml

It should be noted that the composition of the media provided in Table 2 is 30 not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of  $10^2$ - $10^5$  cell/ml, preferably  $3 \times 10^2$ -35  $10^4$  cell/ml. The process can be scaled up or down according to needs. The yeast culture is

grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

- 5           The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of about 300 to about 500 MHz, preferably in the range of 340.000 to 435.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 340, 345, 350, 355, 360, 365, 370, 375, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430 or 435 MHz. The field strength of  
10          the EM field(s) is in the range of 10 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM  
15          fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

- Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the  
20          presence of the EM field(s) for an extended period of time (e.g., one or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 140 - 280 hours.

- For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 10-20mV/cm, usually at about 12.5mV/cm is used. After this  
25          first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the EM field strength is increased to a higher level in the range of 50-200 mV/cm, usually to about 125 mV. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C. The culturing process may preferably be conducted  
30          under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the P-decomposing yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature

below about 0°C to 4°C. The P-decomposing yeast cells may also be dried and stored in powder form.

The amount of biologically available phosphorus, such as H<sub>3</sub>PO<sub>4</sub>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, and HPO<sub>4</sub><sup>2-</sup> in the culture can be determined by any methods known in the art, including but 5 not limited to UV absorption spectroscopy. The increase can be calculated by the difference between the total amount of biologically available phosphorus in a culture with activated yeasts and the amount of biologically available phosphorus in the same medium with non-activated yeast. For example, 1 ml of *Saccharomyces cerevisiae* strain AS2.399 (2 to 5 x 10<sup>7</sup> yeasts/ml) is inoculated into 1000 ml of a medium according to Table 2. The culture is 10 incubated at a temperature of 28°C in the presence of a series of 8 EM fields in the order stated: 360 MHz at 14 mV/cm for 5 hours; 365 MHz at 14 mV/cm for 5 hours; 370 MHz at 14 mV/cm for 5 hours; 380 MHz at 14mV/cm for 5 hours; 360 MHz at 130 mV/cm for 30 hours; 365 MHz at 130 mV/cm for 30 hours; 370 MHz at 130 mV/cm for 30 hours; 375 MHz at 130mV/cm for 30 hours. The increase in the amount of biologically available 15 phosphorus was determined to be greater than 330 mg/ml of yeast culture. There was no significant change in the amount of biologically available phosphorus in the control with non-activated yeasts.

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### 5.3. PHOSPHORUS-BALANCING YEAST CELL COMPONENT

The phosphorus-balancing (P-balancing) yeasts of the invention also convert insoluble or biologically unavailable phosphorus-containing substances into soluble biologically available phosphorous compounds. However, the P-balancing yeast is preferably used when the level of phosphorus in the local environment is high. The 25 conversion of insoluble or biologically unavailable phosphorus-containing substances into soluble biologically available phosphorous is sensitive to the level of phosphorus; at about 180 ppm or higher, the conversion is reduced while at about 60 ppm or lower, the conversion is increased.

In the present invention, the P-balancing yeast cells are preferably deployed 30 in biologically fertilizer compositions that include an organic substrate that already contains a relatively significant level of soluble or biologically available phosphorous. For example, poultry manure contains a relatively high level of soluble phosphorus as compared to other kinds of manure.

According to the invention, yeast cells that are capable of P-balancing are 35 prepared by culturing the cells in the presence of an electromagnetic field in an appropriate

culture medium. The frequency of the electromagnetic field for activating or enhancing P-balancing function in yeasts can generally be found in the range of 300 MHz to 500 MHz. After the cells have been cultured for a sufficient period of time, the cells can be tested for their ability to decompose phosphorus-containing substances by methods well known in the  
5 art.

The method of the invention for making the P-balancing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in  
10 combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 1.5%. These carbon sources can be used individually, or  
15 several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, potassium, calcium, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ . Insoluble phosphorus-containing substances in a suitable form are also included  
20 in the media. Non-limiting examples include powder of dried sludge of > 150 mesh. Other insoluble phosphorus-containing substances can also be used either separately or in combination.

Table 3: Composition for a culture medium for P-balancing yeast

Medium Composition	Quantity
Sucrose	15g
$\text{NaCl}$	1.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	3.0g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.3g
$\text{KNO}_3$	0.3g
Yeast extract paste	0.5g
Dried manure, sludge, or garbage	1.2g; Powder of > 150 mesh

Autoclaved water	1000ml
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It should be noted that the composition of the media provided in Table 3 is not intended to be limiting. Various modifications of the culture medium may be made by  
5 those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of  $10^2$ - $10^5$  cell/ml, preferably  $3 \times 10^2$ -  
10  $10^4$  cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series  
15 of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can  
15 each have a frequency in the range of about 300 to about 500 MHz, or preferably in the range of 380.000 to 485.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 380, 385, 390, 395, 400, 402, 405, 410, 415, 420, 422, 425, 430, 432, 435, 440, 445, 450, 455, 460, 465, 470, 480 or 485 MHz.  
The field strength of the EM field(s) is in the range of 90 to 300 mV/cm. If a series of EM  
20 fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical  
25 number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7 or 8 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is  
30 generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 230 - 480 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 50-150mV/cm, usually at about 100 mV is used. After this first period of culture, the yeast cells are further incubated under substantially the same  
35 conditions for another period, except that the EM field strength is increased to a higher level

in the range of 200-300 mV/cm, usually to about 250mV/cm. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8  
5 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the P-balancing yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The P-balancing yeast cells may also be dried and stored in  
10 powder form.

The amount of biologically available phosphorus, such as H<sub>3</sub>PO<sub>4</sub>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, and HPO<sub>4</sub><sup>2-</sup>, in the culture can be determined by any methods known in the art, including but not limited to UV absorption spectroscopy. The increase can be calculated by the difference between the total amount of biologically available phosphorus in a culture with activated  
15 yeasts and the amount of phosphorus in the same medium with non-activated yeast. For example, 1 ml of *Saccharomyces cerevisiae* strain AS2.628 (2 to 5 x 10<sup>7</sup> yeasts/ml) is inoculated into 1000 ml of a medium containing 200 mg/l of H<sub>3</sub>PO<sub>4</sub>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>2-</sup>. The culture is incubated at a temperature of 28°C in the presence of a series of 8 EM fields in the order stated: 385 MHz at 99 mV/cm for 12 hours; 415 MHz at 99 mV/cm for 12  
20 hours; 440 MHz at 99 mV/cm for 12 hours; 460 MHz at 99 mV/cm for 12 hours; 385 MHz at 250 mV/cm for 48 hours; 415 MHz at 250 mV/cm for 48 hours; 440 MHz at 250 mV/cm for 24 hours; 460 MHz at 250mV/cm for 24 hours. The increase in the amount of biologically available phosphorus was determined to be greater than 24%. The control did not show any significant change in the amount of biologically available phosphorus.

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#### 5.4. POTASSIUM-DECOMPOSING YEAST CELL COMPONENT

The potassium compound-decomposing (K-decomposing) yeasts of the invention converts insoluble potassium-containing substances, such as potassium mica, into soluble potassium so that they become available to plants.

30 In the present invention, the ability of a plurality of yeast cells to decompose insoluble potassium-containing substances is activated or enhanced, and the resulting K-decomposing yeast cells can be used as a component of the biological fertilizer compositions of the invention.

According to the present invention, yeast cells that are capable of  
35 K-decomposing are prepared by culturing the cells in the presence of an electromagnetic

field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing K-decomposition in yeasts can generally be found in the range of 100 MHz -300MHz. After the yeast cells have been cultured for a sufficient period of time, the cells can be tested for their ability to decompose potassium-containing substances by 5 methods well known in the art.

The method of the invention for making the K-decomposing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in 10 combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 1.5%. These carbon sources can be used individually, or 15 several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ . Insoluble potassium-containing substances in a suitable form are 20 also included in the media. Non-limiting examples include powder of potassium mica of  $\geq$  200 mesh. Other insoluble potassium-containing substances can also be used either separately or combined.

Table 4: Composition for a culture medium for K-decomposing yeast

	Medium Composition	Quantity
25	Sucrose	15g
	NaCl	1.2g
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
30	$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	3.0g
	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.3g
	$(\text{NH}_4)_2\text{HPO}_4$	0.3g
	Yeast extract paste	0.5g
35	Potassium mica	1.0g, Powder of $>$ 200 mesh

Medium Composition	Quantity
Dried manure, sludge, or garbage	1.2-3g, Powder of > 150 mesh
Autoclaved water	1000ml

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It should be noted that the composition of the media provided in Table 4 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

10

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of  $10^2$ - $10^5$  cell/ml, preferably  $3 \times 10^2$ - $10^4$  cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

15

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of about 100 to about 300 MHz, preferably in the range of 190.000 to 285.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, or 285 MHz. The field strength of the EM field(s) is in the range of 10 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

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Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., one or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 140 - 280 hours.

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For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 10-20mV/cm, usually at about 125mV/cm is used. After this

first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the EM field strength is increased to a higher level in the range of 50-200 mV/cm, usually to about 125 mV/cm. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to 5 conduct the process at 25° to 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the K-decomposing yeast cells may be 10 recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The K-decomposing yeast cells may also be dried and stored in powder form.

Any methods known in the art can be used to test the cultured yeast cells for their ability to decompose insoluble potassium-containing substances. For example, 1 ml of 15 *Saccharomyces cerevisiae* strain AS2.631 (2 to 5 x 10<sup>7</sup> cells/ml) was inoculated into 1000 ml of a medium according to Table 4. The culture was incubated at a temperature at 28°C in the presence of a series of 8 EM fields in the order stated: 210 MHz at 14 mV/cm for 5 hours; 235 MHz at 14 mV/cm for 5 hours; 245 MHz at 14 mV/cm for 5 hours; 255 MHz at 14mV/cm for 5 hours; 210 MHz at 120 mV/cm for 30 hours; 235 MHz at 120 mV/cm for 20 30 hours; 245 MHz at 120 mV/cm for 30 hours; 255 MHz at 120mV/cm for 30 hours. A control was set up which contained non-activated cells of the same strain of yeasts. The amount of biologically available potassium K<sup>+</sup> in the culture can be determined by any methods known in the art, including but not limited to flame spectroscopy and/or atomic absorption spectrometry. The increase in potassium is calculated by the difference between 25 the quantity of potassium in the medium of Table 4 after culturing and the basal level of potassium in the medium prior to culturing. The increase in the amount of biologically available potassium was determined to be greater than 120 mg/ml of cultured yeast cells. There was no significant change in the amount of potassium available in the control.

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### 5.5. COMPLEX CARBON-DECOMPOSING YEAST CELL COMPONENT

The carbon-decomposing (C-decomposing) yeast of the invention converts complex, high molecular weight, carbon compounds and materials, in particular, complex carbohydrates, such as cellulose and lignin, into simple carbohydrates, such as pentoses and

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hexoses. Such simple carbohydrates are utilized by other yeast cells in the local environment to support their growth and activities.

In the present invention, the ability of yeast to decompose complex carbon compounds efficiently is activated or enhanced, and the resulting C-decomposing yeast cells 5 can be used as a component of the biological fertilizer composition of the invention.

According to the present invention, yeast cells that are capable of C-decomposition are prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for C-decomposition in yeasts can generally be found in the range of 1000 MHz -1200 MHz.  
 10 After the yeast cells have been cultured for a sufficient period of time, the cells can be tested for their ability to decompose complex carbon compounds by methods well known in the art.

The method of the invention for making the C-decomposing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by 15 the yeast cells. Complex carbon-containing substances such as cellulose, lignin, coal powder, etc., in a suitable form can be used as sources of carbon in the culture medium. The exact quantity of the carbon source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of simple carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably 20 between about 0.1% and 1%, and most preferably about 0.5%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{CaCO}_3$ , 25  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

Table 5: Composition for a culture medium for C-decomposing yeasts

Medium Composition	Quantity
Cellulose	3.0g; Powder of > 100 mesh
Dried manure, sludge, or garbage	5g; Powder of > 150 mesh
NaCl	0.6g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.3g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	1.5g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.4g

(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	0.3g
Yeast extract paste	0.5g
K <sub>2</sub> HPO <sub>4</sub>	0.5g
5 Autoclaved water	1000ml

It should be noted that the composition of the media provided in Table 5 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale 10 of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10<sup>2</sup>-10<sup>5</sup> cell/ml, preferably 3x10<sup>2</sup>-10<sup>4</sup> cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series 15 of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of about 1000 to about 1200 MHz, preferably in the 20 range of 1050.000 to 1160.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 1050, 1055, 1060, 1065, 1070, 1075, 1080, 1085, 1090, 1095, 1100, 1105, 1110, 1115, 1120, 1125, 1130, 1135, 1140, 1145, 1150, 1155, or 1160 MHz. The field strength of the EM field(s) is in the range of 10 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different 25 frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is 30 preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., one or more weeks), it is 35 generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 140 - 280 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 10-20mV, usually at about 12.5mV/cm is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the EM field strength is increased to a higher level

5 in the range of 100-200 mV/cm, usually to about 125 mV/cm. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C.. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional

10 means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the C-decomposing yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The C-decomposing yeast cells may also be dried and stored in powder form.

15 Any methods known in the art can be used to test the cultured yeast cells for their ability to decompose complex-carbon containing substances. For example, a change in the chemical oxygen demand (COD) of a sample can be used as an indication of the change in the concentration of complex-carbon containing substances in the sample. For example, 1 ml of the *Saccharomyces cerevisiae* strain AS2.982 (2 to 5 x 10<sup>7</sup> yeasts/ml) is inoculated

20 into 30 ml of a medium according to Table 5. The culture is incubated at a temperature in the range of 20-28°C for in the presence of a series of 8 EM fields in the order stated: 1050 MHz at 16 mV/cm for 5 hours; 1070 MHz at 16 mV/cm for 5 hours; 1090 MHz at 16 mV/cm for 5 hours; 1110 MHz at 16 mV/cm for 5 hours; 1050 MHz at 125 mV/cm for 30 hours; 1070 MHz at 125 mV/cm for 30 hours; 1090 MHz at 125 mV/cm for 30 hours; 1110

25 MHz at 125 mV/cm for 30 hours. After activation, based on a change in COD, the amount of carbohydrates in the culture was estimated to be greater than 330 mg/ml of yeast culture. There was no significant change in the COD of the control culture.

Alternatively, the amount of simple carbohydrates in the culture can then be determined by any methods known in the art, including but not limited to biochemical reactions, chromatography and molecular fluorescence spectroscopy.

### 5.6. GROWTH FACTORS PRODUCING YEAST CELL COMPONENT

The growth factors producing (GF-producing) yeast of the present invention produces many vitamins and other nutrients, such as but not limited to, vitamin B-1, riboflavin (vitamin B-2), vitamin B-12, niacin (B-3), pyridoxine (B-6), pantothenic acid 5 (B-5), folic acid, biotin, para-aminobenzoic acid, choline, inositol, in such amounts that can support the growth of other yeast strains.

The ability of yeast to overproduce growth factors is activated or enhanced by methods of this invention, and the resulting GF-producing yeast cells are included as a component of the biological fertilizer composition of the invention.

10 According to the present invention, yeast cells that are capable of overproducing growth factors are prepared by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing GF-production in yeasts can generally be found in the range of 1300 MHz -1500 MHz. After the yeast cells have been cultured for a 15 sufficient period of time, the cells can be tested for their ability to produce growth factors by methods well known in the art.

The method of the invention for making the GF-producing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, 20 fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% 25 and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $\text{NH}_4\text{NO}_3$ ,  $\text{K}_2\text{HPO}_4$ , 30  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

Table 6: Composition for a culture medium for GF-producing yeasts

Medium Composition	Quantity
Starch	8.0g; Powder of > 120 mesh

35

Medium Composition	Quantity
NaCl	0.3g
MgSO <sub>4</sub> •7H <sub>2</sub> O	0.2g
CaCO <sub>3</sub> •5H <sub>2</sub> O	0.5g
CaSO <sub>4</sub> •2H <sub>2</sub> O	0.2g
NH <sub>4</sub> NO <sub>3</sub>	0.3g
K <sub>2</sub> HPO <sub>4</sub>	0.8g
Autoclaved water	1000ml

It should be noted that the composition of the media provided in Table 6 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10<sup>2</sup>-10<sup>5</sup> cell/ml, preferably 3x10<sup>2</sup>-10<sup>4</sup> cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of about 1300 to about 1500 MHz, preferably in the range of 1340.000 to 1440.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 1340, 1345, 1350, 1355, 1360, 1365, 1370, 1375, 1380, 1385, 1390, 1395, 1400, 1405, 1410, 1415, 1420, 1425, 1430, 1435, or 1440 MHz. The field strength of the EM field(s) is in the range of 20 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7 or 8 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., one or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the  
5 presence of the EM field or EM fields for a total of about 140 - 280 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 20-40mV/cm, usually at about 25mV/cm is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the  
10 range of 100-200 mV/cm, usually to about 125 mV. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means  
15 known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the GF-producing yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The GF-producing yeast cells may also be dried and stored in powder form.

20 Any methods known in the art can be used to test the cultured yeast cells for their ability to overproduce growth factors, including but not limited to high performance liquid chromatography (HPLC). For example, 1 ml of activated or non-activated *Saccharomyces cerevisiae* strain AS2.413 (2 to 5 x 10<sup>7</sup> yeasts/ml) was inoculated into 1000 ml of a medium according to Table 6. The culture was incubated at a temperature of 28°C  
25 in the presence of a series of 8 EM fields in the order stated: 1340 MHz at 28 mV/cm for 5 hours; 1350 MHz at 28 mV/cm for 5 hours; 1380 MHz at 28 mV/cm for 5 hours; 1390 MHz at 28mV/cm for 5 hours; 1340 MHz at 135 mV/cm for 30 hours; 1350 MHz at 135 mV/cm for 30 hours; 1380 MHz at 135 mV/cm for 30 hours; 1390 MHz at 135 mV/cm for 30 hours. The amount of growth factors produced can be calculated by the difference between  
30 the total amount of vitamin B1, B2, B6, and B12 in a culture with activated or non-activated yeasts and the total amount of the same growth factors in the same medium without yeast. The increase in the amount of growth factors was determined to be greater than 350 mg/ml of activated yeast culture. There was no significant change in the total amount of growth factors in the control culture.

### 5.7. ATP-PRODUCING YEAST CELL COMPONENT

The ATP-producing yeast of the present invention is capable of overproducing ATP in such amounts that can support the growth of other yeasts in the biological fertilizer compositions.

- 5 In the present invention, the ability of yeast to overproduce ATP is activated or enhanced, and the resulting ATP-producing yeast cells can be used as a component of the biological fertilizer compositions of the invention.

According to the present invention, yeast cells that are capable of enhanced ATP-production are prepared by culturing the cells in the presence of an electric field in an 10 appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing ATP-production in yeasts can generally be found in the range of 1600 MHz - 1800 MHz. After sufficient time is given for the cells to grow, the cells can be tested for their enhanced ability to produce ATP by methods well known in the art.

The method of the invention for making the ATP-producing yeast cells is 15 carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other 20 ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media 25 are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

Table 7: Composition for a culture medium for ATP-producing yeasts

30 Medium Composition	Quantity
Starch	10.0g, > 120 mesh
NaCl	0.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
35 $\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.8g

	CaSO <sub>4</sub> •2H <sub>2</sub> O	0.2g
	NH <sub>4</sub> NO <sub>3</sub>	0.2g
	K <sub>2</sub> HPO <sub>4</sub>	0.5g
5	Autoclaved water	1000ml

It should be noted that the composition of the media provided in Table 7 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale 10 of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10<sup>2</sup>-10<sup>5</sup> cell/ml, preferably 3x10<sup>2</sup>-10<sup>4</sup> cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series 15 of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of about 1600 to about 1800 MHz, preferably in the 20 range of 1630.000 to 1730.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 1630, 1635, 1640, 1645, 1650, 1655, 1660, 1665, 1670, 1675, 1680, 1685, 1690, 1695, 1700, 1705, 1710, 1715, 1720, 1725, or 1730 MHz. The field strength of the EM field(s) is in the range of 20 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different 25 frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is 30 preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., one or more weeks), it is 35 generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 160 - 300 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 20-40mV/cm, usually at about 30mV/cm is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the 5 range of 100-200 mV/cm, usually to about 150 mV/cm. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional 10 means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the ATP-producing yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The ATP-producing yeast cells may also be dried and stored in powder form.

15 Any methods known in the art can be used to test the cultured yeast cells for their ability to overproduce ATP, including but not limited to HPLC. For example, 1 ml of the activated yeast culture (2 to 5 x 10<sup>7</sup> yeasts/ml) was inoculated into 1000 ml of a medium according to Table 7. The culture was incubated at a temperature of 28°C in the presence of a series of 8 EM fields in the order stated: 1635 MHz at 29 mV/cm for 10 hours; 1655 20 MHz at 29 mV/cm for 10 hours; 1675 MHz at 29 mV/cm for 10 hours; 1695 MHz at 29mV/cm for 10 hours; 1635 MHz at 150 mV/cm for 30 hours; 1655 MHz at 150 mV/cm for 30 hours; 1675 MHz at 150 mV/cm for 30 hours; 1695 MHz at 150mV/cm for 30 hours. The amount of ATP produced can be calculated by the difference between the total amount of ATP in a culture with yeasts and the amount of ATP in the same medium without yeast. 25 Using activated *Saccharomyces cerevisiae* strain AS2.536, the amount of ATP in the culture was determined to be 170 mg/ml of yeast culture.

### 5.8. PATHOGEN-SUPPRESSING YEAST CELL COMPONENT

30 The present invention also provides yeast cells that are capable of suppressing the proliferation of pathogenic microorganisms that are present in the materials used in the organic substrate component of the biological fertilizer. Typically, due to an abundance of nutrients present in the organic substrate material for such pathogenic microorganisms, the numbers of pathogens increase rapidly over a period of time. 35 However, in the presence of the pathogen-suppressing yeasts of the invention, the numbers

of pathogens in the organic substrate material remains unchanged, or decreases over time. Without being bound by any theory or mechanism, the inventor believes that the presence of the pathogen-suppressing yeasts in the organic substrate material creates an environment that is unfavorable for the growth of pathogenic microorganisms.

5 According to the invention, the ability of yeasts to affect/control the numbers of pathogens is activated or enhanced by culturing the yeasts in the presence of an electromagnetic field. The resulting pathogen-suppressing yeast cells are used as a component in the biological fertilizer compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the  
10 ability of yeasts to control the numbers of pathogenic microorganisms can generally be found in the range of 30 MHz to 50 MHz. After sufficient time is given for the yeast cells to grow, the cells can be tested for their ability to affect/control the number of pathogens by methods well known in the art.

The method of the invention for making pathogen-suppressing yeast cells is  
15 carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other  
20 ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media  
25 are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

Table 8: Composition for a culture medium for Pathogen-Suppressing yeasts

30 Medium Composition	Quantity
Soluble Starch	8.0g
Sucrose	5g
NaCl	0.2g
35 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g

Medium Composition	Quantity
CaCO <sub>3</sub> •5H <sub>2</sub> O	0.5g
CaSO <sub>4</sub> •2H <sub>2</sub> O	0.2g
5 Peptone	1.5g
K <sub>2</sub> HPO <sub>4</sub>	0.5g
Autoclaved water	400ml
manure, sludge, or garbage extract	600ml

10       The manure, sludge, or garbage extract for the culture medium is prepared by incubating 500g of fresh poultry manure, cattle manure, swine manure, sludge, or garbage in about 600ml of warm water (at 35°C to 40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. It should be noted that the composition of the media provided in Table 8 is not intended to be limiting. Various modifications of the culture  
 15      medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

20       The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10<sup>2</sup>-10<sup>5</sup> cell/ml, preferably 3x10<sup>2</sup>-10<sup>4</sup> cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

25       The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of about 30.000 to about 50.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 MHz. The field strength of the EM field(s) is in the range of 0.5 to 200 mV/cm, preferably 10 to 180 mV/cm. If a series of EM fields are applied, the EM fields can each have a different  
 30      frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is  
 35

preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., one or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 144 - 272 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 10-30mV/cm, usually at about 25mV/cm is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 100-200 mV/cm, usually to about 150 mV/cm. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the pathogen-suppressing yeast cells may be recovered from the culture by various methods known in the art, and stored at about 0°C to 4°C. The pathogen-suppressing yeast cells may also be dried and stored in powder form.

The ability of the pathogen-suppressing yeasts to control the numbers of pathogens can be determined by any methods known in the art for enumerating microorganisms, such as optical density, plating out dilutions on solid media for counting, or counting individual cells under a microscope. Stains may be applied to distinguish or identify different strains or species of microorganisms present in a sample, or to determine their viability. When a range of pathogenic microorganisms are expected to be affected by the pathogen-suppressing yeasts, the numbers of more than one representative species of pathogenic microorganisms can be monitored to assess the performance of the pathogen-suppressing yeasts.

For example, samples of organic substrate material containing a known concentration of pathogenic microorganisms are cultured under the same conditions for a same period of time in the presence of different concentrations of pathogen-suppressing yeasts, and as negative control, the same strain of yeasts that have not been treated according to the culturing methods of the invention. A sample without any added yeast may

also be included to determine the growth of pathogens under normal circumstances. The numbers of pathogens before and after the culture period are determined and compared.

A one liter culture containing at least  $10^{10}$  cells of a pathogenic microorganism per ml is prepared. One ml of activated yeast cells (containing 2 to  $5 \times 10^7$  yeasts per ml) is added to the one liter culture of pathogenic microorganism and incubated at 30°C for 24 hours. Controls are included which contained non-activated yeast cells or no yeasts. The numbers of microorganisms in the respective cultures are then determined and compared. The following are several examples in which a particular species of pathogenic bacteria was studied.

10 Using cells of *Saccharomyces cerevisiae* strain IFFI1037 which had been cultured in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 hours; 36 MHz at 26 mV/cm for 12 hours; 43 MHz at 26 mV/cm for 12 hours; 47 MHz at 26 mV/cm for 12 hours; 30 MHz at 150 mV/cm for 24 hours; 36 MHz at 150 mV/cm for 24 hours; 43 MHz at 150 mV/cm for 24 hours; 47 MHz at 150mV/cm for 24 hours. The number of *Staphylococcus aureus* in a sample was reduced by more than 2.7% relative to the control with no yeasts. There was no significant change in the number of pathogens in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1021 which had been cultured in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 hours; 36 MHz at 26 mV/cm for 12 hours; 42 MHz at 26 mV/cm for 12 hours; 49 MHz at 26 mV/cm for 12 hours; 30 MHz at 150 mV/cm for 24 hours; 36 MHz at 150 mV/cm for 24 hours; 42 MHz at 150 mV/cm for 24 hours; 49 MHz at 150mV/cm for 24 hours. The number of *Diplococcus pneumoniae* in a sample was reduced by more than 2.8% relative to the control with no yeasts. There was no significant change in the number of pathogens in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1051 which had been cultured in the presence of a series of 8 EM fields in the order stated: 35 MHz at 26 mV/cm for 12 hours; 39 MHz at 26 mV/cm for 12 hours; 43 MHz at 26 mV/cm for 12 hours; 47 MHz at 26 mV/cm for 12 hours; 35 MHz at 150 mV/cm for 24 hours; 39 MHz at 150 mV/cm for 24 hours; 43 MHz at 150 mV/cm for 24 hours; 47 MHz at 150mV/cm for 24 hours. The number of *Bacillus anthracis* in a sample was reduced by more than 3.1% relative to the control with no yeasts. There was no significant change in the number of pathogens in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1331 which had been cultured in the presence of a series of 8 EM fields in the order stated: 33 MHz at 26 mV/cm

for 12 hours; 36 MHz at 26 mV/cm for 12 hours; 45 MHz at 26 mV/cm for 12 hours; 47 MHz at 26 mV/cm for 12 hours; 33 MHz at 150 mV/cm for 24 hours; 36 MHz at 150 mV/cm for 24 hours; 45 MHz at 150 mV/cm for 24 hours; 47 MHz at 150mV/cm for 24 hours. The number of *Mycobacterium tuberculosis* in a sample was reduced by more than 5 2.9% relative to the control with no yeasts. There was no significant change in the number of pathogens in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1345 which had been cultured in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 hours; 34 MHz at 26 mV/cm for 12 hours; 38 MHz at 26 mV/cm for 12 hours; 49 10 MHz at 26 mV/cm for 12 hours; 30 MHz at 150 mV/cm for 24 hours; 34 MHz at 150 mV/cm for 24 hours; 38 MHz at 150 mV/cm for 24 hours; 49 MHz at 150mV/cm for 24 hours. The number of *Escherichia coli* in a sample was reduced by more than 48% relative to the control with no yeasts. There was no significant change in the number of pathogens in the control containing non-activated cells.

15 Using cells of *Saccharomyces cerevisiae* strain IFFI1211 which had been cultured in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 hours; 33 MHz at 26 mV/cm for 12 hours; 36 MHz at 26 mV/cm for 12 hours; 38 MHz at 26 mV/cm for 12 hours; 30 MHz at 150 mV/cm for 24 hours; 33 MHz at 150 mV/cm for 24 hours; 36 MHz at 150 mV/cm for 24 hours; 38 MHz at 150mV/cm for 24 20 hours. The number of *Salmonella* species bacteria in a sample was reduced by more than 66% relative to the control with no yeasts. There was no significant change in the number of pathogens in the control containing non-activated cells.

25 **5.9. YEAST CELL COMPONENT THAT DECOMPOSES  
UNDESIRABLE CHEMICALS**

The present invention further provides yeast cells that are capable of degrading undesirable chemicals, such as antibiotics, that are typically found in manure or sludge.

30 According to the invention, the ability of yeasts to degrade antibiotics is activated or enhanced by culturing the yeasts in the presence of an electromagnetic field. The resulting yeast cells can be used as a component in the biological fertilizer compositions of the invention.

35 The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade undesirable chemicals, in particular antibiotics, can generally be

found in the range of about 70 MHz to about 100 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose antibiotics by methods well known in the art. Antibiotics degraded by the yeasts of the invention include but are not limited to molecules within the families of beta-lactams,

- 5 tetracyclines, polypeptides, glycopeptides, aminoglycosides, and macrolides.

The method of the invention for making antibiotics-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in 10 combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or 15 several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

20

Table 9: Composition for a culture medium for yeasts that degrade undesirable chemicals

Medium Composition	Quantity
Soluble Starch	8.0g, > 120 mesh
25 Sucrose	5g
NaCl	0.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
$\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	0.5g
30 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.2g
Peptone	1.5g
$\text{K}_2\text{HPO}_4$	0.5g
Autoclaved water	1000ml
35 manure, sludge, or garbage extract	600ml

35

The manure, sludge, or garbage extract for the culture medium is prepared by incubating 500g of fresh poultry, cattle, swine manure, sludge, or garbage in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. It should be noted that the composition of the media provided in Table 5 9 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of  $10^2$ - $10^5$  cell/ml, preferably  $3 \times 10^2$ -10 $^4$  cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

- 15        The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 70.000 to 100.000 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 100 MHz. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm.
- 20      If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., one or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 180 - 328 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an initial field strength in the range of 40-60mV/cm, usually at about 50mV is used. After this first 35 period of culture, the yeast cells are further incubated under substantially the same

conditions for another period, except that the amplitude is increased to a higher level in the range of 100-250 mV/cm, usually to about 200 mV/cm. The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C. The culturing process may preferably be conducted

5 under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C

10 to 4°C. The recovered yeast cells may also be dried and stored in powder.

To determine the activity of the activated yeast cells towards an antibiotic compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the antibiotic compound in a test sample at various time point and under different incubation conditions. For example, a known amount of an antibiotic (up to 100

15 mg per liter) is added to 10 liter of an aqueous extract of the manure. Then, 0.1 ml each of activated and non-activated yeasts (at least 10<sup>7</sup> cells/ml) are added to the 10 liter samples containing the antibiotics, and incubated for 24 hours at 28°C. A control is also included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics

remaining in the extracts are determined and compared by performing HPLC on samples of

20 the extracts.

Using cells of *Saccharomyces cerevisiae* strain AS2.293 which had been cultured in the presence of a series of 8 EM fields in the order stated: 77 MHz at 48 mV/cm for 15 hours; 83 MHz at 48 mV/cm for 15 hours; 90 MHz at 48 mV/cm for 15 hours; 96

25 MHz at 48mV/cm for 15 hours; 77 MHz at 200 mV/cm for 30 hours; 83 MHz at 200 mV/cm for 30 hours; 90 MHz at 200 mV/cm for 30 hours; 96 MHz at 200 mV/cm for 30 hours. The amount of penicillin G in a sample was reduced by more than 23% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1063 which had been cultured in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 hours; 73 MHz at 48 mV/cm for 15 hours; 88 MHz at 48 mV/cm for 15 hours; 98

30 MHz at 48mV/cm for 15 hours; 70 MHz at 200 mV/cm for 30 hours; 73 MHz at 200 mV/cm for 30 hours; 88 MHz at 200 mV/cm for 30 hours; 98 MHz at 200 mV/cm for 30 hours. The amount of chlorotetracycline in a sample was reduced by more than 31%

relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1221 which had been cultured in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 hours; 74 MHz at 48 mV/cm for 15 hours; 88 MHz at 48 mV/cm for 15 hours; 98 MHz at 48mV/cm for 15 hours; 70 MHz at 200 mV/cm for 30 hours; 74 MHz at 200 mV/cm for 30 hours; 88 MHz at 200 mV/cm for 30 hours; 98 MHz at 200 mV/cm for 30 hours. The amount of oxytetracycline in a sample was reduced by more than 28% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1340 which had been cultured in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 hours; 73 MHz at 48 mV/cm for 15 hours; 77 MHz at 48 mV/cm for 15 hours; 88 MHz at 48mV/cm for 15 hours; 71 MHz at 200 mV/cm for 30 hours; 73 MHz at 200 mV/cm for 30 hours; 77 MHz at 200 mV/cm for 30 hours; 88 MHz at 200 mV/cm for 30 hours. The amount of doxycycline in a sample was reduced by more than 33% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1215 which had been cultured in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 hours; 75 MHz at 48 mV/cm for 15 hours; 82 MHz at 48 mV/cm for 15 hours; 85 MHz at 48mV/cm for 15 hours; 70 MHz at 200 mV/cm for 30 hours; 75 MHz at 200 mV/cm for 30 hours; 82 MHz at 200 mV/cm for 30 hours; 85 MHz at 200 mV/cm for 30 hours. The amount of tetracycline in a sample was reduced by more than 26% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1213 which had been cultured in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 hours; 73 MHz at 48 mV/cm for 15 hours; 80 MHz at 48 mV/cm for 15 hours; 96 MHz at 48mV/cm for 15 hours; 70 MHz at 200 mV/cm for 30 hours; 73 MHz at 200 mV/cm for 30 hours; 80 MHz at 200 mV/cm for 30 hours; 96 MHz at 200 mV/cm for 30 hours. The amount of streptomycin in a sample was reduced by more than 31% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1206 which had been cultured in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 hours; 78 MHz at 48 mV/cm for 15 hours; 86 MHz at 48 mV/cm for 15 hours; 98 MHz at 48mV/cm for 15 hours; 71 MHz at 200 mV/cm for 30 hours; 78 MHz at 200 mV/cm for 30 hours; 86 MHz at 200 mV/cm for 30 hours; 98 MHz at 200 mV/cm for 30 hours. The amount of kanamycin in a sample was reduced by more than 25% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1211 which had been 10 cultured in the presence of a series of 8 EM fields in the order stated: 73 MHz at 48 mV/cm for 15 hours; 79 MHz at 48 mV/cm for 15 hours; 88 MHz at 48 mV/cm for 15 hours; 98 MHz at 48mV/cm for 15 hours; 73 MHz at 200 mV/cm for 30 hours; 79 MHz at 200 mV/cm for 30 hours; 88 MHz at 200 mV/cm for 30 hours; 98 MHz at 200 mV/cm for 30 hours. The amount of erythromycin in a sample was reduced by more than 27% relative to 15 the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

Using cells of *Saccharomyces cerevisiae* strain IFFI1210 which had been cultured in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 hours; 77 MHz at 48 mV/cm for 15 hours; 84 MHz at 48 mV/cm for 15 hours; 93 20 MHz at 48mV/cm for 15 hours; 70 MHz at 200 mV/cm for 30 hours; 77 MHz at 200 mV/cm for 30 hours; 84 MHz at 200 mV/cm for 30 hours; 93 MHz at 200 mV/cm for 30 hours. The amount of spiramycin in a sample was reduced by more than 22% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

25 Using cells of *Saccharomyces cerevisiae* strain IFFI1260 which had been cultured in the presence of a series of 8 EM fields in the order stated: 75 MHz at 48 mV/cm for 15 hours; 78 MHz at 48 mV/cm for 15 hours; 81 MHz at 48 mV/cm for 15 hours; 95 MHz at 48mV/cm for 15 hours; 75 MHz at 200 mV/cm for 30 hours; 78 MHz at 200 mV/cm for 30 hours; 81 MHz at 200 mV/cm for 30 hours; 95 MHz at 200 mV/cm for 30 30 hours. The amount of bacitracin in a sample was reduced by more than 17% relative to the control with no yeasts. There was no significant change in the concentration of the antibiotic in the control containing non-activated cells.

### 5.10. ODOR-REDUCING YEAST CELL COMPONENT

- The present invention also provides yeast cells that are capable of reducing the odor of manure, sludge, or garbage. Without being bound by any theory, the inventor believes that the yeast cells of the invention are capable of reducing the odor of manure, 5 sludge, or garbage by modifying or decomposing known and unknown compounds in such organic materials that are malodorous. However, it is not necessary to demonstrate that such compounds have been decomposed. It is sufficient so long as the odor is reduced as determined subjectively by a panel of subjects, after the yeast cells of the invention have been used.
- 10 According to the present invention, yeast cells that are capable of reducing the odor of organic materials are prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing this ability in yeasts can generally be found in the range of about 2160 to about 2380 MHz. After sufficient time is given for the yeast 15 cells to grow, the yeast cells can be tested for their ability to reduce the odor of organic materials by methods well known in the art.

The method of the invention for making the odor-reducing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, 20 fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% 25 and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{K}_2\text{HPO}_4$ , 30  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

Table 10: Composition for a culture medium for yeasts that reduce odor

Medium Composition	Quantity
manure, sludge, garbage	100g

35

	NaCl	0.2g
	MgSO <sub>4</sub> •7H <sub>2</sub> O	0.2g
	CaCO <sub>3</sub> •5H <sub>2</sub> O	0.5g
5	CaSO <sub>4</sub> •2H <sub>2</sub> O	0.2g
	K <sub>2</sub> HPO <sub>4</sub>	0.5g
	Autoclaved water	900ml

It should be noted that the composition of the media provided in Table 10 is  
10 not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10<sup>2</sup>-10<sup>5</sup> cell/ml, preferably 3x10<sup>2</sup>-  
15 10<sup>4</sup> cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

20 The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 2160.000 to 2380.000 MHz, and preferably in the ranges of 2160 to 2250 MHz or 2280 to 2380 MHz. For example and without being limited by such examples, each EM field can have a frequency at about 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240,  
25 2245, 2250, 2280, 2285, 2290, 2295, 2300, 2305, 2315, 2320, 2325, 2330, 2335, 2340, 2345, 2350, 2355, 2360, 2365, 2370, 2375, or 2380 MHz. The field strength of the EM field(s) is in the range of 0.5 to 320 mV/cm, preferably 30 to 300 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength  
30 within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, or 8 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the 5 presence of the EM field or EM fields for a total of about 80-320 hours.

The process of the invention is carried out at temperatures ranging from about 23° to 30°C; however, it is preferable to conduct the process at 25° to 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 mol/m<sup>3</sup>. The oxygen 10 level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The recovered yeast cells may also be dried and stored in powder form.

15 Any methods known in the art can be used to test the cultured yeast cells for their ability to reduce the odor of organic materials. The amount of malodorous chemicals such as hydrogen sulfide, ammonia, indole, p-cresol, skatol, and organic acids present in a test sample of organic material can be determined by any methods known in the art, including but not limited to gas phase chromatography, olfactometry, mass spectrometry, or 20 the use of an odor panel.

To determine the activity of the activated yeast cells towards a malodorous compound, methods well known in the art, such as HPLC or mass spectrometry (e.g., VG micromass), can be used to measure the amounts of the malodorous compound in a test sample at various time point and under different incubation conditions. For example, a 25 known amount of a malodorous compound (up to 100 mg per liter) is added to 10 liter of an aqueous extract of manure. Then, 0.1 ml of activated and non-activated yeasts (at least 10<sup>7</sup> cells/ml) are added to the 10 liter samples containing the antibiotics, and incubated for 24 hours at 28°C. A control is also included which does not contain any yeast cells. After 24 hours, the amounts of the malodorous compounds remaining in the extracts are determined 30 and compared.

Accordingly, the odor caused by hydrogen sulfide and other related sulfur-containing or sulphydryl (SH-) containing molecules can be reduced by yeasts cultured in the presence of an EM field that is in the range of 2160.000 to 2250.000. Using cells of *Saccharomyces cerevisiae* strain AS2.559 which had been cultured in the presence of a 35 series of four EM fields in the order stated: 2165 MHz at 240 mV/cm for 20 hours; 2175

MHz at 240 mV/cm for 20 hours; 2200 MHz at 240 mV/cm for 20 hours; and 2235 MHz at 240 mV/cm for 20 hours, the amount of hydrogen sulfide in a sample was reduced by more than 13% relative to the control containing no yeasts. There was no significant reduction in the malodorous compound in the sample containing non-activated yeasts.

5       The odor caused by ammonia and related NH-containing compounds can be reduced by yeasts cultured in the presence of an EM field that is in the range of 2160.000 to 2250.000. Using cells of *Saccharomyces cerevisiae* strain AS2.423 which had been cultured in the presence of a series of four EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hours; 2175 MHz at 250 mV/cm for 20 hours; 2210 MHz at 250 mV/cm for 10 hours; and 2245 MHz at 250 mV/cm for 10 hours, the amount of ammonia in a sample was reduced by more than 11% relative to the control containing no yeasts. There was no significant reduction in the malodorous compound in the sample containing non-activated yeasts.

15      The odor caused by indole and other related molecules, such as skatol, can be reduced by yeasts cultured in the presence of an EM field that is in the range of 2160.000 to 2250.000. Using cells of *Saccharomyces cerevisiae* strain AS2.612 which had been cultured in the presence of a series of four EM fields in the order stated: 2165 MHz at 240 mV/cm for 40 hours; 2180 MHz at 240 mV/cm for 20 hours; 2200 MHz at 240 mV/cm for 40 hours; and 2220 MHz at 240 mV/cm for 20 hours, the amount of indole in a sample was 20 reduced by more than 15% relative to the control containing no yeasts. There was no significant reduction in the malodorous compound in the sample containing non-activated yeasts.

25      The odor caused by organic acids (such as formic acid, acetic acid, propanoic acid, butyric acid, and other volatile fatty acids) can be reduced by yeasts cultured in the presence of an EM field that is in the range of 2280.000 to 2380.000. Using cells of *Saccharomyces cerevisiae* strain AS2.53 which had been cultured in the presence of a series of four EM fields in the order stated: 2315 MHz at 290 mV/cm for 30 hours; 2335 MHz at 290 mV/cm for 10 hours; 2355 MHz at 290 mV/cm for 20 hours; and 2375 MHz at 290 mV/cm for 10 hours, the amount of acetic acid in a sample was reduced by more than 19% relative to the control containing no yeasts. There was no significant reduction in the malodorous compound in the sample containing non-activated yeasts.

30      The odor caused by methylamine, dimethylamine, trimethylamine, and other aliphatic substituted amines can be reduced by yeasts cultured in the presence of an EM field that is in the range of 2160.000 to 2250.000. Using cells of *Saccharomyces cerevisiae* strain AS2.541 which had been cultured in the presence of a series of four EM fields in the

order stated: 2160 MHz at 250 mV/cm for 20 hours; 2190 MHz at 250 mV/cm for 10 hours; 2210 MHz at 250 mV/cm for 40 hours; and 2250 MHz at 250 mV/cm for 40 hours, the amount of methyl-substituted amines in a sample was reduced by more than 23% relative to the control containing no yeasts. There was no significant reduction in the 5 malodorous compound in the sample containing non-activated yeasts.

The odor caused by p-cresol and related compounds can be reduced by yeasts cultured in the presence of an EM field that is in the range of 2280.000 to 2380.000. Using cells of *Saccharomyces cerevisiae* strain AS2.163 which had been cultured in the presence of a series of four EM fields in the order stated: 2300 MHz at 98 mV/cm for 20 hours; 2370 10 MHz at 98 mV/cm for 15 hours; 2300 MHz at 250 mV/cm for 20 hours; and 2370 MHz at 250 mV/cm for 30 hours, the amount of p-cresol in a sample was reduced by more than 23% relative to the control containing no yeasts. There was no significant reduction in the malodorous compound in the sample containing non-activated yeasts.

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### 5.11. FORMATION OF SYMBIOSIS-LIKE RELATIONSHIPS

In another embodiment of the present invention, yeast cells with the newly activated or enhanced ability to (1) fix nitrogen, (2) decompose phosphorus-containing minerals or compounds, (3) balance phosphorus compounds, (4) decompose insoluble 20 potassium-containing minerals or compounds, and (5) decompose complex carbon compounds as described in Sections 5.1-5.5 are combined and cultured so that they form a symbiosis-like relationship whereby they can grow together without substantially relying on outside supplies of biological available nitrogen, phosphorus, potassium, and carbon nutrients. The nutrients needed for growth are supplied by the respective nutrient-producing 25 yeast strain within the fertilizer composition by converting biologically-unavailable nutrients from various sources into available nutrients. The activity of each of the yeast strains in producing the respective types of nutrient relates in part to the needs of other yeast cells as well as the plants. As a result, soluble, biologically-available nutrients will be converted when needed, thereby avoiding excess losses due to, for example, leaching.

30 The optional process which can be used to improve the performance of the biological fertilizer is described as follows. At least four strains of yeasts prepared according to Sections 5.1-5.5 are mixed and cultured in the presence of an electromagnetic field in an appropriate liquid medium. The medium contains nitrogen, phosphorus, potassium, and carbon nutrients in biologically unavailable forms. As non-limiting 35 examples, atmospheric nitrogen is used as the source of nitrogen nutrient, powder of

phosphate rock is used as the source of phosphorus nutrient, powder of potassium mica is used as the source of potassium nutrient, and powdered cellulose is used as the source of complex carbon nutrient. Other forms of insoluble phosphorus- and potassium-containing substances and complex carbon compounds may also be used in place of or in combination 5 with any of the above-identified minerals as sources of phosphorus, potassium, and carbon nutrients. Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $\text{CaCO}_3$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

10 Table 11: Composition for a culture medium for formation of symbiosis-like relation

Medium Composition	Quantity
NaCl	0.5g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.4g
15 $\text{CaCO}_3 \cdot 5\text{H}_2\text{O}$	3.0g
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.3g
Yeast extract paste	0.3g
Potassium mica	1.2g; Powder of > 200 mesh
20 Rock phosphate	1.2g; Powder of > 200 mesh
Cellulose	5.0g; Powder of > 200 mesh
Autoclaved water	1000ml

It should be noted that the composition of the media provided in Table 11 is 25 not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m<sup>3</sup>, preferably 0.4 30 mol/m<sup>3</sup>. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The process is initiated in sterilized medium by inoculating typically about 20ml of each inoculum of the four strains of yeast cells, each at a

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cell density of about  $10^8$  cell/ml. The optional process can be scaled up or down according to needs.

The yeast culture is grown for 12-72 hours, preferably for about 48 hours, in the presence of four independent electromagnetic fields. The electromagnetic fields, which  
5 can be applied by a variety of means, each has the following respective frequencies: (1) in the range of about 840 to about 916 MHz for nitrogen-fixing; (2) in the range of about 300 to about 500 MHz for phosphorus-decomposing or phosphorus balancing; (3) in the range of about 100 to about 300 MHz for potassium-decomposing; and (4) in the range of about 1000 to about 1200 MHz for complex carbon-decomposing. Generally, the yeast cells are  
10 subjected to an EM field strength in the range from 5 mV/cm to 160 mV/cm per complete cycle. Using an exemplary apparatus as depicted in Figure 2, the output amplitude of the EM waves used are in the range of 0-3000mV, preferably 20-1800mV. The amplitude of each electromagnetic field is repeatedly cycled between 0mV to 3000mV, preferably  
15 between 20mV to 1800mV, in steps of 1mV at a rate of about two to about ten minutes per complete cycle.

### 5.12. SOIL ADAPTATION

The yeast cells of the invention must also be able to grow and perform their  
20 respective functions in various types of soils. The ability of the yeast cells to survive and grow can be enhanced by adapting the yeast cells of the invention to a particular soil condition.

In another embodiment of the invention, yeast cells prepared according to any one of Sections 5.1-5.10 can be cultured separately or in a mixture in a solid or semi-  
25 solid medium containing soil from one or more soil sources. This optional process which can be used to improve the performance of the biological fertilizer described by way of an example as follows.

A suspension containing 10ml of yeasts at a density of  $10^6$  cell/ml is mixed with a  $1000\text{cm}^3$  of the soil medium. The process can be scaled up or down according to  
30 needs. The mixture of yeast and soil is cultured for about 48-96 hours, preferably for about 48 hours, in the presence of an electromagnetic field. The electromagnetic field, which can be applied by a variety of means, has a frequency that, depending on the function of the yeasts, corresponds to one of the frequencies described in Sections 5.1-5.10. Generally, the yeast cells are subjected to an EM field strength in the range from 60mV/cm to 250mV/cm  
35 in this process.

The culture is incubated at temperatures that cycle between about 4°C to about 48°C. For example, in a typical cycle, the temperature of the culture may start at 35-48°C and be kept at this temperature for about 1-2 hours, then adjusted up to 42-45°C and kept at this temperature for 1-2 hours, then adjusted to 26-30°C and kept at this temperature for about 2-4 hours, and then brought down to 5-10°C and kept at this temperature for about 1-2 hours, and then the temperature may be raised again to 35-45°C for another cycle. The cycles are repeated until the process is completed. After the last temperature cycle is completed, the temperature of the culture is lowered to 3-4°C and kept at this temperature for about 5-6 hours. After adaptation, the yeast cells may be isolated and recovered from the medium by conventional methods, such as filtration. The adapted yeast cells can be stored under 4°C. An exemplary set-up of the culture process is depicted in Figure 3.

### **5.13. SEPARATION OR ENRICHMENT OF YEAST CELLS**

Yeast cells that have been adapted to form a symbiosis-like relationship according to Section 5.11 can be separated or enriched in such a way that each strain of yeast cells keep their acquired or enhanced functions. Separation of yeast cells is carried out according to methods described in U.S. Patent No. 5,578,486 and Chinese patent publication CN 1110317A which are incorporated herein by reference in its entirety. The same frequency used for activating the yeast cells may be used during the separation process. The separated yeast cells can then be dried, and stored.

### **5.14. MANUFACTURE OF THE BIOLOGICAL FERTILIZERS**

In addition to the yeast cell components, an organic substrate component, and optionally inorganic materials are also included in the biological fertilizer compositions of the invention. The preparation of manure, sludge, garbage and such materials as well as the steps involved in the manufacture of the biological fertilizer compositions are described below.

30

#### **5.14.1. Preparation of the Organic and Inorganic Substrate Components**

Any types of manure, sludge, or garbage can be used in the biological fertilizer compositions of the present invention. Mixtures of different kinds of manure (poultry, cattle, swine) can also be used. Organic compounds present in the manure, sludge, or garbage are decomposed by the yeasts of the invention. Depending on the type of

- manure, sludge, garbage, in addition to nitrogen, it may contain an useful amount of phosphorus (e.g.,  $P_2O_5$ ) and potassium (e.g.,  $K_2O$ ). Nutrient concentrations in poultry, cattle or swine manure, can vary due to the species and breeds, differences in feeding rations, and methods of storage and moisture content. Mixtures of sludge from different sources can
- 5 also be used. Nutrient concentrations in sludge, can vary due to its origin, processing if any, methods of storage and moisture content. Nutrient concentrations in garbage, can vary due to the source (e.g. residential, commercial), geographical location of the sources, differences in processing, and methods of storage, and moisture content. Garbage that contains a high organic content (i.e. greater than 30%) is preferred. Methods known in the art can be
- 10 employed to determine the nutrient value of each batch of organic substrate prior to its use in making a biological fertilizer composition.

Inorganic materials, such as but not limited to phosphate rock and potassium mica, can optionally be included as additional sources of phosphorus and potassium respectively. Other phosphorous- or potassium-containing materials and minerals can also

15 be used. These inorganic compounds are decomposed by K-decomposing and P-decomposing yeast cells into biologically available potassium and biologically available phosphorus that can be used by the growing plants as well as the yeast cells in the fertilizer.

Any inorganic material may be used in combination with the organic substrates in the present invention. Alternatively, the inorganic ingredients may be omitted,

20 or substituted by another if it is deemed desirable by the particular application. For example, phosphate rock can be omitted if poultry manure is used which contains a relatively high level of biologically available phosphorus.

The manure, sludge, or garbage is preferably dried to a moisture content of  $\leq$  5%. Both the dried manure, sludge, or garbage and optional inorganic substrate

25 components in the present invention are ground into suitable forms and sizes before incorporated into the fertilizer. Typically, the organic material and/or inorganic material is conveyed into a crusher where it is broken up into pieces of  $\leq 5$  cm in diameter. Any conventional crusher or equivalent machines can be used for this purpose. The pieces are then transferred to a grinder by any conveying means and ground to a powder of  $\geq 150$

30 mesh. Any grinder that allows fine grinding can be used for this purpose. The powder is then conveyed to an appropriate storage tank for storage until use with other components of the fertilizer. A schematic illustration of the grinding process is shown in Figs. 4 and 5.

#### 5.14.2. Fermentation Process Using Growth Factor-Producing Yeast

In the present invention, the preparation of GF-producing yeast is carried out in a fermentation process using as seed the activated yeast strain as described in Section 5.6. A schematic of the fermentation process is illustrated in Fig. 6.

The fermentation medium is prepared according to a ratio of 2.5 liters of water per kilogram of starch. Clean water, preferably water free of any microorganisms, is used to prepare the fermentation medium. The fermentation is carried out at a temperature between 20-30°C, preferably between 25-28°C, in a clean environment and in a space where there are no strong sources of electromagnetic fields, such as power lines and power generators. Any equipments that contact the fermentation broth, including reactors, pipelines, and stirrers, must be thoroughly cleaned before each use. The fermentation process normally lasts about 48-72 hours at 28-30°C when at least 90% of the fermentation substrate is fermented. Fermentation is preferably conducted under semi-aerobic conditions or conditions in which the oxygen level is about 20-60% of the maximal soluble oxygen concentration. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling. After fermentation, the cell counts should reach about  $2 \times 10^{10}$  cells/ml. The fermentation broth is kept at a temperature in the range of 15-28°C and must be used within 24 hours. Alternatively, the GF-producing yeasts can be drained, dried and stored in powder form.

#### 20           **5.14.3. Fermentation Process Using ATP-Producing Yeast**

In the present invention, the preparation of ATP-producing yeast is carried out by a fermentation process using as seed the adapted yeast strain as described in Section 5.7. A schematic of the fermentation process is illustrated in Fig. 6.

The fermentation medium is prepared according to a ratio of 2.5 liters of water per kilogram of starch. Clean water, preferably water free of any microorganisms, most preferably autoclaved water, is used to prepare the fermentation media. The fermentation is carried out at a temperature between 20-30°C, preferably between 25-28°C, in a clean environment and in a space where there are no strong sources of electromagnetic fields, such as power lines and power generators. Any equipments that contact the fermentation broth, including reactors, pipelines, and stirrers, must be thoroughly cleaned before each use. The fermentation process normally lasts about 48-72 hours, depending on the fermentation temperature. Preferably at the end of the process at least 90% of the fermentation substrate is fermented. Fermentation is preferably conducted under semi-aerobic conditions or conditions in which the oxygen level is about 20-60% of the maximal soluble oxygen concentration. The oxygen level can be controlled by any conventional

means known to one skilled in the art, including but not limited to stirring and/or bubbling. After fermentation, the cell counts should reach about  $2 \times 10^{10}$  cells/ml. The fermentation broth is kept at a temperature in the range of 15-28°C and must be used within 24 hours. Alternatively, the ATP-producing yeasts can be drained, dried and stored in powder form.

5

#### **5.14.4. Preparation of Mixture of Raw Materials**

The organic substrate (manure, sludge, or garbage) and the optional inorganic raw materials are mixed in exemplary proportions as shown in Table 12. Appropriate amount of organic and inorganic materials prepared according to Section 5.10.1 and starch are conveyed to a mixer. Any conventional mixer, such as but not limited a rotary drum mixer, can be used. The mixing tank is rotated constantly so that powders of manure, sludge, or garbage, and starch are mixed evenly. The mixture is then conveyed to a storage tank. The procedure for mixing manure, sludge, or garbage and inorganic substrate material is illustrated in FIG. 7.

15

Table 12 Ratio of raw materials

Material	Percentage	Requirement
Powder of manure, sludge, or garbage	58-63%	$\geq 150$ mesh, water content $\leq 5\%$
Powder of inorganic materials	20%	$\geq 150$ mesh, water content $\leq 3\%$
Starch	10-15%	regular starch powder, water content $\leq 8\%$

25

#### **5.14.5. Preparation of Yeast Mixture**

If no inorganic materials is used, the proportion of manure, sludge, or garbage can be increased up to 80%. A yeast mixture is prepared in the exemplary proportions as shown in Table 13. Appropriate amounts of the nine yeast strains in dried powder form prepared according to Section 5.1-5.10 are conveyed to a mixing tank. The yeasts are allowed to mix for about 10-20 minutes. The mixture is then transferred to a storage tank. Any equipments used for mixing yeasts, including the mixing tank and the storage tank, must be thoroughly cleaned, preferably sterilized, before each use. The yeast mixture is stored at a temperature below 20°C and must be used within 24 hours. The

procedure for mixing yeasts is illustrated in FIG. 8. Alternatively, the mixture of nine yeasts can be dried and stored in powder form.

Table 13 Ratio of microorganisms

	Yeast	Quantity	Percentage (dry weight)	Note
5	Nitrogen-fixing yeast	1.0-2.0kg	0.1-0.2%	Dry yeast powder
10	Phosphorus-decomposing yeast	1.0-2.0kg	0.1-0.2%	Dry yeast powder
15	Potassium-decomposing yeast	1.0-2.0kg	0.1-0.2%	Dry yeast powder
20	Carbon-decomposing yeast	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	Pathogen-suppressing yeast	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	Chemical-decomposing yeast	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	Odor-reducing yeast	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	Growth factor-producing yeast	25L	1%	Yeast fermentation broth
	ATP-producing yeast	75L	3%	Yeast fermentation broth

#### 5.14.6. Manufacture of Biological Fertilizer

The biological fertilizer of the present invention is produced by mixing the yeast mixture of Section 5.14.5 and the mixture of the organic and inorganic materials of Section 5.14.1 at a ratio according to Table 14. For example, the yeasts and the organic substrate (poultry manure, swine manure, cattle manure, sludge, or garbage) and inorganic materials are conveyed to a granulator to form granules. The granules of the fertilizer are then dried in a two-stage drying process. During the first drying stage, the fertilizer is dried in a first dryer at a temperature not exceeding 65°C for a period of time not exceeding 10 minutes so that yeast cells quickly become dormant. The fertilizer is then sent to a second dryer and dried at a temperature not exceeding 70°C for a period of time not exceeding 30 minutes to further remove water. After the two stages, the water content should be lower than 5%. It is preferred that the temperatures and drying times be adhered to in both drying stages so that yeast cells do not lose their vitality and functions. The fertilizer is then cooled to room temperature. The fertilizer may also be screened in a separator so that fertilizer

granules of a preferred size are selected. Any separator, such as but not limited to a turbo separator with adjustable speed and screen sizes, can be used. The fertilizer of the selected size is then sent to a bulk bag filler for packing.

The production process is illustrated in Figs. 9-11. Fig. 9 is a schematic illustration of the procedure for producing the fertilizer from its components. Fig. 10 is a schematic illustration of the drying process. Fig. 11 is a schematic illustration of the cooling and packing process.

Table 14 Composition of the biological fertilizer (for one metric ton of fertilizer)

10	Quantity	Percentage (dry weight)	Note
Mixture of raw materials	952-956kg	95.2-95.4%	Dry weight
Mixture of yeasts	100L	4.4-4.8%	Dry weight

15

## 6. EXAMPLE

The following examples demonstrate the manufacture of an exemplary biological fertilizer composition of the present invention. These examples represent 20 preferred embodiments of the present invention.

**6.1 Biological Fertilizer Composition Comprising Poultry Manure**  
*Saccharomyces cerevisiae* strains having accession numbers AS2.628, AS2.631, AS2.982, AS2.413 and AS2.536 are used to prepare the yeast cell components of 25 the biological fertilizer composition. All were deposited in China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms. Yeast strain AS2.628 is cultured according to the method described in Section 5.1 for nitrogen-fixation; and to the method described in Section 5.3 for P-balancing. Yeast strain AS2.631 is cultured according to the method described in Section 30 5.4 for K-decomposition. Yeast strain AS2.982 is cultured according to the method described in Section 5.5 for C-decomposition. Yeast strain AS2.413 is cultured according to the method described in Section 5.6 for production of growth factor. Yeast strain AS2.536 is cultured according to the method described in Section 5.7 for ATP production. Yeast strain IFFI1221 is cultured according to the method described in Section 5.8 for 35 suppressing growth of pathogens. Yeast strain IFFI1293 is cultured according to the

method described in Section 5.9 for degrading undesirable chemicals. Yeast strain AS2.607 is cultured according to the method described in Section 5.10 for odor reduction.

The poultry manure in powder form is prepared according to Section 5.14.1.

The production of growth factor-producing yeast is carried out in a

- 5 fermentation process using as seed the activated yeast strain AS2.413 as described in Section 5.6. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of  
 10 medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol}/\text{m}^3$  in a clean environment where there were no sources of electromagnetic fields. After about 48 hours of fermentation, the concentration of yeast cells reached about  $2\times10^{10}$  cells/ml.

- 15 The production of ATP-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.536 as described in Section 5.7. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The  
 20 fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol}/\text{m}^3$  for about 56 hours in a clean environment where there were no sources of electromagnetic fields. After fermentation, the cell counts reached about  $2\times10^{10}$  cells/ml.

The mixture of raw materials is prepared according to Table 15 and the procedure in Section 5.14.4.

25

Table 15 Ratio of raw materials

Material	Percentage	Requirement
Powder of dried poultry manure	80.3%	$\geq 150$ mesh, water content $\leq 5\%$
Starch	15%	regular starch powder, water content $\leq 8\%$

30 The yeast mixture is prepared according to Table 15 and the procedure  
 35 described in Section 5.14.5.

Table 16 Ratio of yeasts (for 1 metric ton of fertilizer)

Yeast	Quantity	Percentage (dry weight)	Note
5 Nitrogen-fixing yeast AS2.628	2.0kg	0.2%	Dry yeast powder
	2.0kg	0.2%	Dry yeast powder
	2.0kg	0.2%	Dry yeast powder
10 Carbon-decomposing yeast AS2.982	2.0kg	0.2%	Dry yeast powder
	1.0-2.0kg	0.1-0.2%	Dry yeast powder
15 Pathogen-suppressing yeast IFFI1221	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	1.0-2.0kg	0.1-0.2%	Dry yeast powder
20 Odor-reducing yeast AS2.607	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	25L	1%	Yeast fermentation broth
ATP producing yeast AS2.536	75L	3%	Yeast fermentation broth

The biological fertilizer is produced by mixing the yeast mixture, the organic materials at a ratio according to Table 17. The mixed yeasts and organic materials were conveyed to a granulator to form granules. The granules of the fertilizer were then dried in a two stage drying process. During the first drying stage, the fertilizer is dried in a first dryer at a temperature not exceeding  $60\pm2^{\circ}\text{C}$  for a period of 5 minutes so that yeast cells quickly became dormant. The fertilizer is then sent to a second dryer and dried at a temperature not exceeding  $65\pm2^{\circ}\text{C}$  for a period of 8 minutes to further remove water. The fertilizer is then cool to room temperature. The fertilizer is then sent to a bulk bag filler for packing.

Table 17 Fertilizer composition (for 1 metric ton of fertilizer)

	Quantity	Percentage (dry weight)	Note
5	Raw material mixture	949kg	94.9% Dry weight
	Yeast mixture	100L	5.1% Dry weight

## 6.2 Biological Fertilizer Composition Comprising Cattle manure

*Saccharomyces cerevisiae* strains having accession numbers AS2.628,

10 AS2.399, AS2.631, AS2.982, AS2.413 and AS2.536 are used to prepare the yeast cell components of the biological fertilizer composition. All were deposited in China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms. Yeast strain AS2.628 is cultured according to the method described in Section 5.1 for nitrogen-fixation; and yeast strain AS2.399 to the method described in Section 5.2 for P-decomposition. Yeast strain AS2.631 is cultured according to the method described in Section 5.4 for K-decomposition. Yeast strain AS2.982 is cultured according to the method described in Section 5.5 for C-decomposition. Yeast strain AS2.413 is cultured according to the method described in Section 5.6 for production of growth factor. Yeast strain AS2.536 is cultured according to the method described in Section 5.7 for ATP production. Yeast strain IFFI1308 is cultured according to the method described in Section 5.8 for suppressing growth of pathogens. Yeast strain IFFI1210 is cultured according to the method described in Section 5.9 for degrading undesirable chemicals. Yeast strain IFFI1213 is cultured according to the method described in Section 5.10 for odor reduction.

25 The cattle manure used in the example contained less than 5% of moisture and was prepared according to Section 5.14.1.

The production of growth factor-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.413 as described in Section 5.6. A schematic of the fermentation process is illustrated in Fig. 6. The 30 fermentation medium is prepared according to a ratio of 2.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4 \text{ mol/m}^3$  in a clean environment where there were no sources of 35 electromagnetic fields. After about 48 hours of fermentation, the concentration of yeast cells reached about  $2\times10^{10}$  cells/ml.

The production of ATP-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.536 as described in Section 5.7. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10  
 5 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol/m}^3$  for about 56 hours in a clean environment where there were no sources of electromagnetic fields. After fermentation, the cell counts reached about  $2\times10^{10}$  cells/ml.  
 10 The mixture of raw materials was prepared according to Table 18 and the procedure in Section 5.14.4.

Table 18 Ratio of raw materials

Material	Percentage	Requirement
Powder of dried cattle manure	80.3%	$\geq 150$ mesh, water content $\leq 5\%$
Starch	15%	regular starch powder, water content $\leq 8\%$

20 The yeast mixture was prepared according to Table 19 and the procedure described in Section 5.14.5.

Table 19 Ratio of yeasts (for 1 metric ton of fertilizer)

Yeast	Quantity	Percentage (dry weight)	Note
Nitrogen-fixing yeast AS2.628	2.0kg	0.2%	Dry yeast powder
Phosphorus-decomposing yeast AS2.399	2.0kg	0.2%	Dry yeast powder
Potassium-decomposing yeast AS2.631	2.0kg	0.2%	Dry yeast powder
Carbon-decomposing yeast AS2.982	2.0kg	0.2%	Dry yeast powder

	Pathogen-suppressing yeast IFFI1308	1.0-2.0kg	0.1-0.2%	Dry yeast powder
5	Chemical-degrading yeast IFFI1210	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	Odor-reducing yeast IFFI1213	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	Growth factor producing yeast AS2.413	25L	1%	Yeast fermentation broth
10	ATP producing yeast AS2.536	75L	3%	Yeast fermentation broth

The biological fertilizer was produced by mixing the yeast mixture and the organic materials at a ratio according to Table 20. The mixed yeasts and organic materials were conveyed to a granulizer to form granules. The granules of the fertilizer were then 15 dried in a two stage drying process. During the first drying stage, the fertilizer was dried in a first dryer at a temperature not exceeding  $60\pm2^{\circ}\text{C}$  for a period of 5 minutes so that yeast cells quickly became dormant. The fertilizer was then sent to a second dryer and dried at a temperature not exceeding  $65\pm2^{\circ}\text{C}$  for a period of 8 minutes to further remove water. The fertilizer was then cool to room temperature, and was then sent to a bulk bag filler for 20 packing.

Table 20 Fertilizer composition (for 1 metric ton of fertilizer)

	Quantity	Percentage (dry weight)	Note
25	Raw material mixture	949kg	Dry weight
	Yeast mixture	100L	Dry weight

### 30 6.1 Biological Fertilizer Composition Comprising Swine Manure

*Saccharomyces cerevisiae* strains having accession numbers AS2.628, AS2.399, AS2.631, AS2.982, AS2.413 and AS2.536 are used to prepare the yeast cell components of the biological fertilizer composition. All were deposited in China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture 35 Collection of Microorganisms. Yeast strain AS2.628 is cultured according to the method described in Section 5.1 for nitrogen-fixation; and yeast strain AS2.399 to the method

described in Section 5.2 for P-decomposition. Yeast strain AS2.631 is cultured according to the method described in Section 5.4 for K-decomposition. Yeast strain AS2.982 is cultured according to the method described in Section 5.5 for C-decomposition. Yeast strain AS2.413 is cultured according to the method described in Section 5.6 for production  
5 of growth factor. Yeast strain AS2.536 is cultured according to the method described in Section 5.7 for ATP production. Yeast strain IFFI1307 is cultured according to the method described in Section 5.8 for suppressing growth of pathogens. Yeast strain IFFI1206 is cultured according to the method described in Section 5.9 for degrading undesirable chemicals. Yeast strain IFFI1052 is cultured according to the method described in Section  
10 5.10 for odor reduction.

Swine manure was used as prepared according to Sections 5.14.1.

The production of growth factor-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.413 as described in Section 5.6. A schematic of the fermentation process is illustrated in Fig. 6. The  
15 fermentation medium is prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol}/\text{m}^3$  in a clean environment where there were no sources of  
20 electromagnetic fields. After about 48 hours of fermentation, the concentration of yeast cells reached about  $2\times10^{10}$  cells/ml.

The production of ATP-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.536 as described in Section 5.7. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is  
25 prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol}/\text{m}^3$  for about 56 hours in a clean environment where there were no sources of  
30 electromagnetic fields. After fermentation, the cell counts reached about  $2\times10^{10}$  cells/ml.

The production of ATP-producing yeast was carried out in a fermentation process using as seed the activated yeast strain AS2.1190 as described in Section 5.7. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium was prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10  
35 kilograms of starch per metric ton of biological fertilizer. The fermentation medium was

inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation was carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol/m}^3$  for about 56 hours in a clean environment where there were no sources of electromagnetic fields. After fermentation, the cell counts reached about  $2\times10^{10}$  cells/ml.

5 The mixture of raw materials was prepared according to Table 21 and the procedure in Section 5.14.5.

Table 21 Ratio of raw materials

Material	Percentage	Requirement
Dried swine manure in powder form	80%	$\geq 150$ mesh, water content $\leq 5\%$
Starch	15%	regular starch powder, water content $\leq 8\%$

10 15 The yeast mixture was prepared according to Table 22 and the procedure described in Section 5.14.5.

Table 22 Ratio of yeasts (for 1 metric ton of fertilizer)

Yeast	Quantity	Percentage (dry weight)	Note
Nitrogen-fixing yeast AS2.628	2.0kg	0.2%	Dry yeast powder
Phosphorus-decomposing yeast AS2.399	2.0kg	0.2%	Dry yeast powder
Potassium-decomposing yeast AS2.631	2.0kg	0.2%	Dry yeast powder
Carbon-decomposing yeast AS2.982	2.0kg	0.2%	Dry yeast powder
Pathogen-suppressing yeast IFFI1307	1.0-2.0kg	0.1-0.2%	Dry yeast powder
Chemical-degrading yeast IFFI1206	1.0-2.0kg	0.1-0.2%	Dry yeast powder
Odor-reducing yeast IFFI1052	1.0-2.0kg	0.1-0.2%	Dry yeast powder

5	Growth factor producing yeast AS2.413	25L	1%	Yeast fermentation broth
	ATP producing yeast AS2.536	75L	3%	Yeast fermentation broth

The biological fertilizer was produced by mixing the yeast mixture and the organic materials at a ratio according to Table 23. The mixed yeasts and organic materials were conveyed to a granulizer to form granules. The granules of the fertilizer were then dried in a two stage drying process. During the first drying stage, the fertilizer was dried in 10 a first dryer at a temperature not exceeding  $60\pm2^{\circ}\text{C}$  for a period of 5 minutes so that yeast cells quickly became dormant. The fertilizer was then sent to a second dryer and dried at a temperature not exceeding  $65\pm2^{\circ}\text{C}$  for a period of 8 minutes to further remove water. The fertilizer was then cool to room temperature. The fertilizer was then sent to a bulk bag filler for packing.

15

Table 23 Fertilizer composition (for 1 metric ton of fertilizer)

	Quantity	Percentage (dry weight)	Note
20	Raw material mixture	949kg	Dry weight
	Yeast mixture	100L	Dry weight (51 kg/ton)

#### 25 6.4 Biological Fertilizer Composition Comprising Sludge

*Saccharomyces cerevisiae* strains having accession numbers AS2.628, AS2.399, AS2.631, AS2.982, AS2.413 and AS2.536 are used to prepare the yeast cell components of the biological fertilizer composition. All were deposited in China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture 30 Collection of Microorganisms. Yeast strain AS2.628 is cultured according to the method described in Section 5.1 for nitrogen-fixation; and yeast strain AS2.399 to the method described in Section 5.2 for P-decomposition. Yeast strain AS2.631 is cultured according to the method described in Section 5.4 for K-decomposition. Yeast strain AS2.982 is cultured according to the method described in Section 5.5 for C-decomposition. Yeast 35 strain AS2.413 is cultured according to the method described in Section 5.6 for production

of growth factor. Yeast strain AS2.536 is cultured according to the method described in Section 5.7 for ATP production. Yeast strain IFFI1301 is cultured according to the method described in Section 5.8 for suppressing growth of pathogens. Yeast strain IFFI1291 is cultured according to the method described in Section 5.9 for degrading undesirable chemicals. Yeast strain IFFI1202 is cultured according to the method described in Section 5.10 for odor reduction.

Dried sludge in powder form was prepared as described in Section 5.14.5.

The production of growth factor-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.413 as described in Section 5.6. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol/m}^3$  in a clean environment where there were no sources of electromagnetic fields. After about 48 hours of fermentation, the concentration of yeast cells reached about  $2\times10^{10}$  cells/ml.

The production of ATP-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.536 as described in Section 5.7. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is prepared according to a ratio of 2.5-3.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol/m}^3$  for about 56 hours in a clean environment where there were no sources of electromagnetic fields. After fermentation, the cell counts reached about  $2\times10^{10}$  cells/ml.

The mixture of raw materials was prepared according to Table 24 and the procedure in Section 5.14.5.

30      Table 24 Ratio of raw materials

Material	Percentage	Requirement
Dried sludge in powder form	80.3%	$\geq 150$ mesh, water content $\leq 5\%$

Starch	15%	regular starch powder, water content ≤ 8%
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The yeast mixture was prepared according to Table 25 and the procedure  
 5 described in Section 5.14.5.

Table 25 Ratio of yeasts (for 1 metric ton of fertilizer)

Yeast	Quantity	Percentage (dry weight)	Note
Nitrogen-fixing yeast AS2.628	2.0kg	0.2%	Dry yeast powder
Phosphorus-decomposing yeast AS2.399	2.0kg	0.2%	Dry yeast powder
Potassium-decomposing yeast AS2.631	2.0kg	0.2%	Dry yeast powder
Carbon-decomposing yeast AS2.982	2.0kg	0.2%	Dry yeast powder
Pathogen-suppressing yeast IFFI1301	1.0-2.0kg	0.1-0.2%	Dry yeast powder
Chemical-degrading yeast IFFI1291	1.0-2.0kg	0.1-0.2%	Dry yeast powder
Odor-reducing yeast IFFI1202	1.0-2.0kg	0.1-0.2%	Dry yeast powder
Growth factor producing yeast AS2.413	25L	1%	Yeast fermentation broth
ATP producing yeast AS2.536	75L	3%	Yeast fermentation broth

The biological fertilizer was produced by mixing the yeast mixture, sludge,  
 30 and any optional inorganic materials at a ratio according to Table 26. The mixed yeasts and sludge were conveyed to a granulator to form granules. The granules of the fertilizer were then dried in a two stage drying process. During the first drying stage, the fertilizer was dried in a first dryer at a temperature not exceeding  $60\pm2^{\circ}\text{C}$  for a period of 5 minutes so that yeast cells quickly became dormant. The fertilizer was then sent to a second dryer and  
 35 dried at a temperature not exceeding  $65\pm2^{\circ}\text{C}$  for a period of 8 minutes to further remove

water. The fertilizer was then cool to room temperature. The fertilizer was then sent to a bulk bag filler for packing.

Table 26 Fertilizer composition (for 1 metric ton of fertilizer)

	Quantity	Percentage (dry weight)	Note
Raw material mixture	949kg	94.9%	Dry weight
Yeast mixture	100L	4.8%	Dry weight

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### 6.5 Biological Fertilizer Composition Comprising Garbage

*Saccharomyces cerevisiae* strains having accession numbers AS2.628, AS2.399, AS2.631, AS2.982, AS2.413 and AS2.536 are used to prepare the yeast cell components of the biological fertilizer composition. All were deposited in China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms. Yeast strain AS2.628 is cultured according to the method described in Section 5.1 for nitrogen-fixation; and yeast strain AS2.399 to the method described in Section 5.2 for P-decomposition. Yeast strain AS2.631 is cultured according to the method described in Section 5.4 for K-decomposition. Yeast strain AS2.982 is cultured according to the method described in Section 5.5 for C-decomposition. Yeast strain AS2.413 is cultured according to the method described in Section 5.6 for production of growth factor. Yeast strain AS2.536 is cultured according to the method described in Section 5.7 for ATP production. Yeast strain IFFI120 is cultured according to the method described in Section 5.8 for suppressing growth of pathogens. Yeast strain IFFI 1059 is cultured according to the method described in Section 5.9 for degrading undesirable chemicals. Yeast strain IFFI 1247 is cultured according to the method described in Section 5.10 for odor reduction.

Garbage was used as the organic substrate which was prepared according to Sections 5.14.1. The garbage used in the example contained at least 30% of organic materials.

The production of growth factor-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.413 as described in Section 5.6. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is prepared according to a ratio of 2.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The

fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol/m}^3$  in a clean environment where there were no sources of electromagnetic fields. After about 48 hours of fermentation, the concentration of yeast 5 cells reached about  $2\times10^{10}$  cells/ml.

The production of ATP-producing yeast is carried out in a fermentation process using as seed the activated yeast strain AS2.536 as described in Section 5.7. A schematic of the fermentation process is illustrated in Fig. 6. The fermentation medium is prepared according to a ratio of 2.5 liters of clean water per kilogram of starch and 10 kilograms of starch per metric ton of biological fertilizer. The fermentation medium is inoculated according to a ratio of 10ml of seed solution per liter of medium. The fermentation is carried out at a temperature of  $28\pm1^{\circ}\text{C}$  and an oxygen concentration of  $0.4\text{mol/m}^3$  for about 56 hours in a clean environment where there were no sources of electromagnetic fields. After fermentation, the cell counts reached about  $2\times10^{10}$  cells/ml.

15 The mixture of raw materials was prepared according to Table 27 and the procedure in Section 5.14.5.

Table 27 Ratio of raw materials

Material	Percentage	Requirement
Dried garbage in powder form	80.3%	$\geq 150$ mesh, water content $\leq 5\%$
Starch	15%	regular starch powder, water content $\leq 8\%$

25 The yeast mixture was prepared according to Table 28 and the procedure described in Section 5.14.5.

Table 28 Ratio of yeasts (for 1 metric ton of fertilizer)

Yeast	Quantity	Percentage (dry weight)	Note
Nitrogen-fixing yeast AS2.628	2.0kg	0.2%	Dry yeast powder
Phosphorus-decomposing yeast AS2.399	2.0kg	0.2%	Dry yeast powder

	Potassium-decomposing yeast AS2.631	2.0kg	0.2%	Dry yeast powder
5	Carbon-decomposing yeast AS2.982	2.0kg	0.2%	Dry yeast powder
	Pathogen-suppressing yeast IFFI 1201	1.0-2.0kg	0.1-0.2%	Dry yeast powder.
10	Chemical-degrading yeast IFFI 1059	1.0-2.0kg	0.1-0.2%	Dry yeast powder
	Odor-reducing yeast IFFI 1247	1.0-2.0kg	0.1-0.2%	Dry yeast powder
15	Growth factor producing yeast AS2.413	25L	1%	Yeast fermentation broth
	ATP producing yeast AS2.536	75L	3%	Yeast fermentation broth

The biological fertilizer was produced by mixing the yeast mixture and the organic materials at a ratio according to Table 29. The mixed yeasts and organic materials were conveyed to a granulator to form granules. The granules of the fertilizer were then dried in a two stage drying process. During the first drying stage, the fertilizer was dried in a first dryer at a temperature not exceeding  $60\pm2^{\circ}\text{C}$  for a period of 5 minutes so that yeast cells quickly became dormant. The fertilizer was then sent to a second dryer and dried at a temperature not exceeding  $65\pm2^{\circ}\text{C}$  for a period of 8 minutes to further remove water. The fertilizer was then cool to room temperature. The fertilizer was then sent to a bulk bag filler for packing.

Table 29 Fertilizer composition (for 1 metric ton of fertilizer)

	Quantity	Percentage (dry weight)	Note
30	Raw material mixture	949kg	94.9% Dry weight
35	Yeast mixture	100L	5.1% Dry weight (51 kg/ton)

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention.

- 5 Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A biological fertilizer composition comprising:
  - (I) an organic substrate;
  - 5 (II) at least one of the following:
    - (a) a first yeast cell component comprising a first plurality of yeast cells that fix nitrogen;
    - (b) a second yeast cell component comprising a second plurality of yeast cells that decompose phosphorus compounds; or
    - 10 (c) a third yeast cell component comprising a third plurality of yeast cells that decompose potassium compounds; and
  - (III) at least one of the following:
    - (d) a fourth yeast cell component comprising a fourth plurality of yeast cells that suppress the growth of pathogenic microorganisms;
    - (e) a fifth yeast cell component comprising a fifth plurality of yeast cells that degrade antibiotics; or
    - (f) a sixth yeast cell component comprising a sixth plurality of yeast cells that reduce the odor of the biological fertilizer composition.
2. The biological fertilizer composition of claim 1, further comprising at least one of the following:
  - (g) a seventh yeast cell component comprising a seventh plurality of yeast cells that convert complex carbon compounds to simple carbohydrates;
  - 25 (h) an eighth yeast cell component comprising an eighth plurality of yeast cells that overproduce growth factors; or
  - (i) a ninth yeast cell component comprising a ninth plurality of yeast cells that overproduce adenosine triphosphate.
3. A biological fertilizer composition comprising:
  - (I) organic substrate;
  - 30 (II) at least one of the following:

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- (a) a first yeast cell component prepared by culturing a first plurality of yeast cells in a first electromagnetic field having a frequency in the range of 840 to 916 MHz and a field strength of 10 to 200 mV/cm;
- 5 (b) a second yeast cell component prepared by culturing a second plurality of yeast cells in a second electromagnetic field having a frequency in the range of 300 to 500 MHz and a field strength of 10 to 300 mV/cm;
- 10 (c) a third yeast cell component prepared by culturing a third plurality of yeast cells in a third electromagnetic field having a frequency in the range of 190 to 285 MHz and a field strength of 10 to 200 mV/cm; and
- (III) at least one of the following:
- 15 (d) a fourth yeast cell component prepared by culturing a fourth plurality of yeast cells in a fourth electromagnetic field having a frequency in the range of 30 to 50 MHz and a field strength of 20 to 200 mV/cm;
- 20 (e) a fifth yeast cell component prepared by culturing a fifth plurality of yeast cells in a fifth electromagnetic field having a frequency in the range of 70 to 100 MHz and a field strength in the range of 40 to 250 mV/cm; and
- 25 (f) a sixth yeast cell component prepared by culturing a sixth plurality of yeast cells in a sixth electromagnetic field having a frequency in the range of 2160 to 2250 MHz and 2280 to 2380 MHz and a field strength in the range of 100 to 300 mV/cm.
4. The biological fertilizer composition of claim 3, further comprising at least one of the following:
- 30 (g) a seventh yeast cell component prepared by culturing a seventh plurality of yeast cells in a fourth electromagnetic field having a frequency in the range of 1050 to 1160 MHz and a field strength of 10 to 200 mV/cm;
- 35 (h) an eighth yeast cell component prepared by culturing an eighth plurality of yeast cells in an eighth electromagnetic

field having a frequency in the range of 1340 to 1440 MHz and a field strength of 20 to 200 mV/cm; and

- (i) a ninth yeast cell component prepared by culturing a ninth plurality of yeast cells in a ninth electromagnetic field having a frequency in the range of 1630 to 1730 MHz and a field strength of 20 to 200 mV/cm.

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5. The biological fertilizer composition of claim 2 or 4 wherein each yeast cell component comprises yeast cells that is from the genus of *Saccharomyces*.

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6. The biological fertilizer composition of claim 2 or 4 wherein each yeast cell component separately comprises cells of a species of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguum*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum* Beijer, *Saccharomyces willianus*, *Saccharomyces ludwigii*, *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

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7. The biological fertilizer composition of claim 2 or 4 wherein each yeast cell component separately comprises cells of a strain of yeast selected from the group consisting of *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, AS2.173, AS2.174, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380, AS2.382, AS2.390, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576, AS2.593, AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190, AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009, IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1043, IFFI 1045, IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 35

1060, IFFI 1063, IFFI 1202, IFFI 1203, IFFI 1206, IFFI 1209, IFFI 1210, IFFI 1211, IFFI  
1212, IFFI 1213, IFFI 1215, IFFI 1220, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI  
1251, IFFI 1270, IFFI 1277, IFFI 1287, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1291, IFFI  
1292, IFFI 1293, IFFI 1297, IFFI 1300, IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI  
5 1309, IFFI 1310, IFFI 1311, IFFI 1331, IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI  
1339, IFFI 1340, IFFI 1345, IFFI 1348, IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1411, IFFI  
1413; *Saccharomyces cerevisiae* Hansen Var. ellipsoideus (Hansen) Dekker, ACCC2043,  
AS2.2, AS2.3, AS2.8, AS2.53, AS2.163, AS2.168, AS2.483, AS2.541, AS2.559, AS2.606,  
AS2.607, AS2.611, AS2.612; *Saccharomyces chevalieri* Guilliermond, AS2.131, AS2.213;  
10 *Saccharomyces delbrueckii*, AS2.285; *Saccharomyces delbrueckii* Lindner var. mongolicus  
Lodder et van Rij, AS2.209, AS2.1157; *Saccharomyces exiguum* Hansen, AS2.349,  
AS2.1158; *Saccharomyces fermentati* (Saito) Lodder et van Rij, AS2.286, AS2.343;  
*Saccharomyces logos* van laer et Denamur ex Jorgensen, AS2.156, AS2.327, AS2.335;  
*Saccharomyces mellis* Lodder et Kreger Van Rij, AS2.195; *Saccharomyces microellipsooides*  
15 Osterwalder, AS2.699; *Saccharomyces oviformis* Osterwalder, AS2.100; *Saccharomyces*  
*rosei* (Guilliermond) Lodder et kreger van Rij, AS2.287; *Saccharomyces rouxii* Boutroux,  
AS2.178, AS2.180, AS2.370, AS2.371; *Saccharomyces sake* Yabe, ACCC2045;  
*Saccharomyces carlsbergensis* Hansen, ACCC2032, ACCC2033, AS2.113, AS2.116,  
AS2.118, AS2.121, AS2.132, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377,  
20 AS2.417, AS2.420, AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605,  
AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Saccharomyces uvarum* Beijer, IFFI  
1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces*  
*willianus* Saccardo, AS2.5, AS2.7, AS2.119, AS2.152, AS2.293, AS2.381, AS2.392,  
AS2.434, AS2.614, AS2.1189; *Saccharomyces* sp., AS2.311; *Saccharomyces ludwigii*  
25 Hansen, ACCC2044, AS2.243, AS2.508; and *Saccharomyces sinenses* Yue, AS2.1395.

8. The biological fertilizer composition of claim 2 or 4 wherein each yeast cell component comprises cells of *Saccharomyces cerevisiae*.

30 9. The biological fertilizer composition of claim 2 or 4 further comprising an inorganic substrate component.

10. The biological fertilizer composition of claim 2 or 4 wherein the inorganic substrate component comprises one or more of rock phosphate, apatite,  
35 phosphorite, sylvinitite, halite, carnalitite, or potassium mica.

11. The biological fertilizer composition of claim 2 which comprises yeast cell components (a) through (f) of claim 1, and yeast cell components (g) thorugh (i) of claim 2.

5 12. The biological fertilizer composition of claim 4 which comprises yeast cell components (a) through (f) of claim 3, and yeast cell components (g) thorugh (i) of claim 4.

13. The biological fertilizer composition of claim 2 or 4 wherein yeast  
10 cell component (a) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.628; yeast cell component (b) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.628; yeast cell component (c) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.631; yeast cell component (d) comprises cells of one or more of the following yeast strain *Saccharomyces cerevisiae* IFFI1037, IFFI1021, IFFI1051, IFFI1331,  
15 IFFI1345, or IFFI1211; yeast cell component (e) comprises cells of one or more of the following yeast strain *Saccharomyces cerevisiae* IFFI1063, IFFI1211, IFFI1340, IFFI1215, IFFI1213, IFFI1206, IFFI1211, IFFI1210, or IFFI1260; yeast cell component (f) comprises cells of one or more of the following yeast strain *Saccharomyces cerevisiae* AS2.559, AS2.423, AS2.612, AS2.53, AS2.541, or AS2.163; yeast cell component (g) comprises cells  
20 of the yeast strain *Saccharomyces cerevisiae* AS2.982; yeast cell component (h) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.413; and yeast cell component (i) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.536.

14. The biological fertilizer composition of claim 11 or 12 wherein yeast  
25 cell component (a) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.628; yeast cell component (b) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.628; yeast cell component (c) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.631; yeast cell component (d) comprises cells of one or more of the following yeast strain *Saccharomyces cerevisiae* IFFI1037, IFFI1021, IFFI1051, IFFI1331,  
30 IFFI1345, or IFFI1211; yeast cell component (e) comprises cells of one or more of the following yeast strain *Saccharomyces cerevisiae* IFFI1063, IFFI1211, IFFI1340, IFFI1215, IFFI1213, IFFI1206, IFFI1211, IFFI1210, or IFFI1260; yeast cell component (f) comprises cells of one or more of the following yeast strain *Saccharomyces cerevisiae* AS2.559, AS2.423, AS2.612, AS2.53, AS2.541, or AS2.163; yeast cell component (g) comprises cells  
35 of the yeast strain *Saccharomyces cerevisiae* AS2.982; yeast cell component (h) comprises

cells of the yeast strain *Saccharomyces cerevisiae* AS2.413; and yeast cell component (i) comprises cells of the yeast strain *Saccharomyces cerevisiae* AS2.536.

15. The biological fertilizer composition of claim 2 or 4, wherein the  
5 pluralities of yeast cells are dried.

16. A method of producing the biological fertilizer composition of claim  
1 or 3, comprising in the order stated:

- 10 (I) preparing a mixture of yeast cells by mixing at least one yeast cell component (a), (b), or (c); with at least one yeast cell component (d), (e), or (f); and  
(II) adding an organic substrate to said mixture of yeast cells to form the biological fertilizer composition.

15 17. A method of producing the biological fertilizer composition of claim  
2 or 4, comprising in the order stated:

- 20 (I) preparing a mixture of yeast cells by mixing at least one yeast cell component (a), (b), or (c); at least one yeast cell component (d), (e), or (f); with at least one yeast cell component (g), (h), or (i); and  
(II) adding an organic substrate to said mixture of yeast cells to form the biological fertilizer composition.

18. The method of claim 16, wherein:  
25 the first yeast cell component of (a) is prepared by culturing a first plurality of yeast cells in a first electromagnetic field or a first series of electromagnetic fields having a frequency in the range of 840 to 916 MHz and a field strength of 10 to 200 mV/cm;  
the second yeast cell component of (b) prepared by culturing a second plurality of yeast cells in a second electromagnetic field or a second series of electromagnetic fields having a frequency in the range of 300 to 500 MHz and a field strength of 10 to 300 mV/cm;  
30 the third yeast cell component of (c) is prepared by culturing a third plurality of yeast cells in a third electromagnetic field or a third series of electromagnetic

fields having a frequency in the range of 190 to 285 MHz and a field strength of 10 to 200 mV/cm;

the fourth yeast cell component of (d) is prepared by culturing a fourth plurality of yeast cells in a fourth electromagnetic field or a fourth series of 5 electromagnetic fields having a frequency in the range of 30 to 50 MHz and a field strength of 20 to 200 mV/cm;

the fifth yeast cell component of (e) is prepared by culturing a fifth plurality of yeast cells in a fifth electromagnetic field or a fifth series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength in the range of 10 40 to 250 mV/cm; and

the sixth yeast cell component of (f) is prepared by culturing a sixth plurality of yeast cells in a sixth electromagnetic field or a sixth series of electromagnetic fields having a frequency in the range of 2160 to 2250 MHz and 2280 to 2380 MHz and a field strength in the range of 100 to 300 mV/cm.

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19. The method of claim 17, wherein:

the seventh yeast cell component of (g) is prepared by culturing a seventh plurality of yeast cells in a seventh electromagnetic field or a seventh series of electromagnetic fields having a frequency in the range of 1050 to 1160 MHz and a field 20 strength of 10 to 200 mV/cm;

the eighth yeast cell component of (h) is prepared by culturing an eighth plurality of yeast cells in an eighth electromagnetic field or a eighth series of electromagnetic fields having a frequency in the range of 1340 to 1440 MHz and a field strength of 20 to 200 mV/cm; and

25 the ninth yeast cell component of (i) is prepared by culturing a ninth plurality of yeast cells in a ninth electromagnetic field or a ninth series of electromagnetic fields having a frequency in the range of 1630 to 1730 MHz and a field strength of 20 to 200 mV/cm.

30 20. The method of claim 16, wherein said mixture of yeast cells in step (II) is added to the organic substrate, starch, and an inorganic substrate component.

21. The method of claim 17, wherein said mixture of yeast cells in step (II) is added to the organic substrate, starch, and an inorganic substrate component.

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22. The method of claim 16, further comprising in the order stated:
- (III) drying said biological fertilizer composition at a temperature not exceeding 65°C for a period such that the yeast cells become dormant;
- 5 (IV) drying said biological fertilizer composition at a temperature not exceeding 70°C for a period such that the water content is less than 5%;
- (V) cooling said biological fertilizer composition to ambient temperature; and
- 10 (VI) forming granules of said biological fertilizer composition.
23. The method of claim 17, further comprising in the order stated:
- (III) drying said biological fertilizer composition at a temperature not exceeding 65°C for a period such that the yeast cells become dormant;
- 15 (IV) drying said biological fertilizer composition at a temperature not exceeding 70°C for a period such that the water content is less than 5%;
- (V) cooling said biological fertilizer composition to ambient temperature; and
- 20 (VI) forming granules of said biological fertilizer composition.
24. A method for enhancing plant growth comprising growing the plant  
25 in the presence of a biological fertilizer composition of claim 2 or 4.
26. The method of claim 23 wherein the biological fertilizer composition is applied to soil at the depth of the major root system of the plant.
- 30 27. The method of claim 23 wherein about 600 to 1350 kg/ha of the biological fertilizer composition is used.
- 28 28. The method of claim 23 wherein the plant is a cereal crop, vegetable crop, fruit crop, flower crop, or grass crops.

28. The method of claim 23 wherein the plant is wheat, barley, corn, soybean, rice, oat, potato, apple, orange, tomato, melon, cherry, lemon, lettuce, carrot, sugar cane, tobacco, or cotton.

5 29. The biological fertilizer composition of claim 1 or 2 wherein the yeast cells of yeast cell component (b) is capable of maintaining a balance of phosphorus compounds.

10 30. The biological fertilizer composition of claim 3 or 4 wherein the yeast cell component (b) is prepared by culturing a second plurality of yeast cells in a second electromagnetic field having a frequency in the range of 380 to 465 MHz and a field strength of 90 to 300 mV/cm.

15 31. The method of claim 18 wherein the frequency of the electromagnetic field(s) for culturing the second yeast component of (b) are in the range of 380 to 465 MHz.

32. The method of claim 19 wherein the frequency of the electromagnetic field(s) for culturing the second yeast component of (b) are in the range of 380 to 465 MHz.

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33. The biological fertilizer composition of claim 1, 2, 3, or 4 wherein the organic substrate is poultry manure, cattle manure, swine manure, sludge, or garbage.

25 34. The biological fertilizer composition of claim 8 wherein the organic substrate is poultry manure, cattle manure, swine manure, sludge, or garbage.

35. The biological fertilizer composition of claim 9 wherein the organic substrate is poultry manure, cattle manure, swine manure, sludge, or garbage.

30 36. The biological fertilizer composition of claim 11 or 12 wherein the organic substrate is poultry manure, cattle manure, swine manure, sludge, or garbage.

37. The method of claim 16 wherein the organic substrate is poultry manure, cattle manure, swine manure, sludge, or garbage.

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38. The method of claim 17 wherein the organic substrate is poultry manure, cattle manure, swine manure, sludge, or garbage.

39. The method of claim 18 wherein the organic substrate is poultry  
5 manure, cattle manure, swine manure, sludge, or garbage.

40. The method of claim 19 wherein the organic substrate is poultry manure, cattle manure, swine manure, sludge, or garbage.

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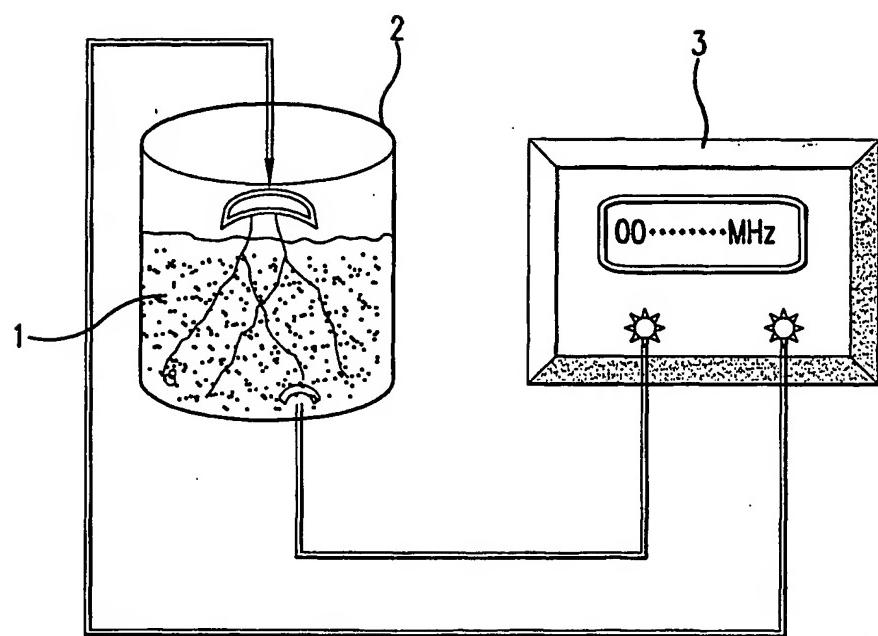


FIG.1

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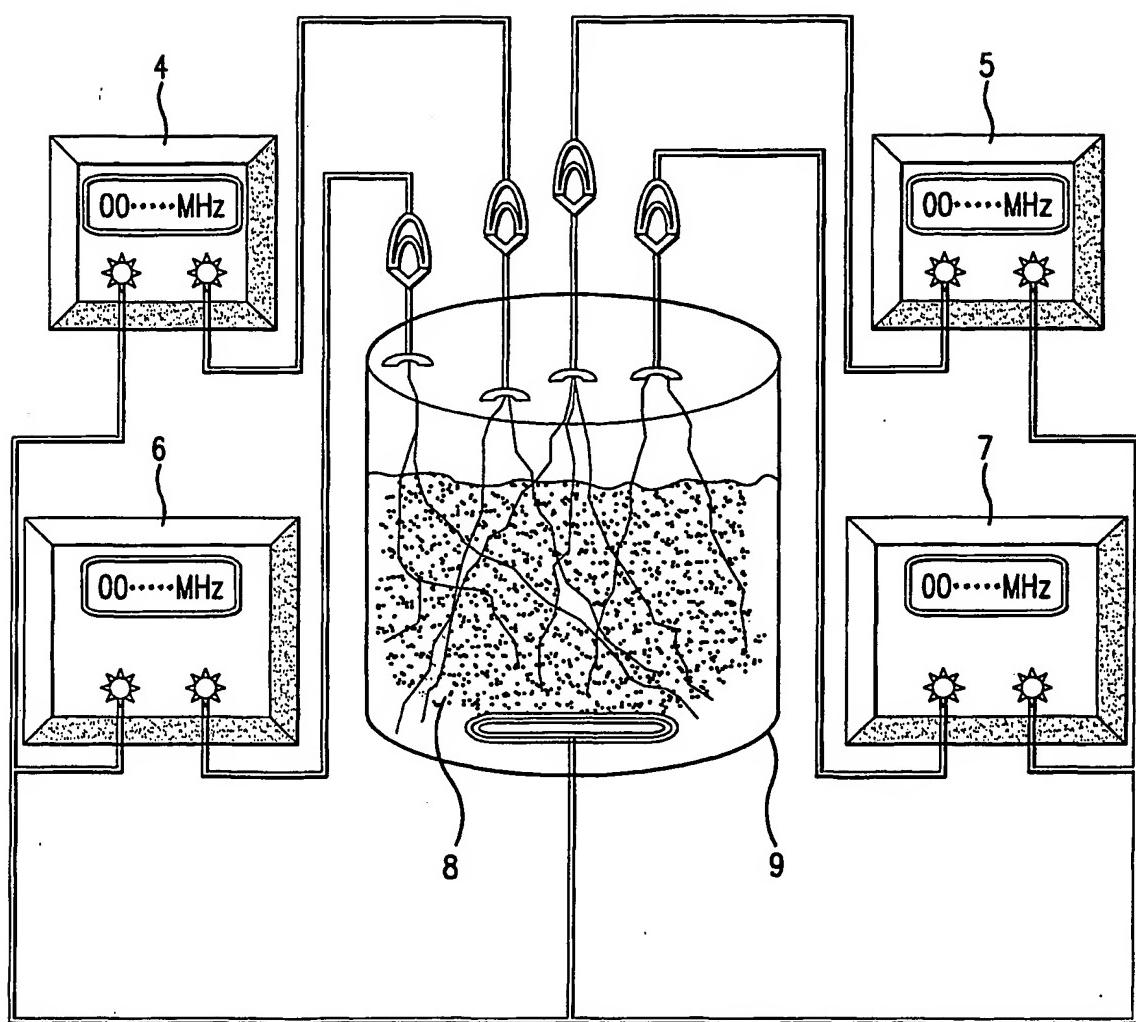


FIG.2

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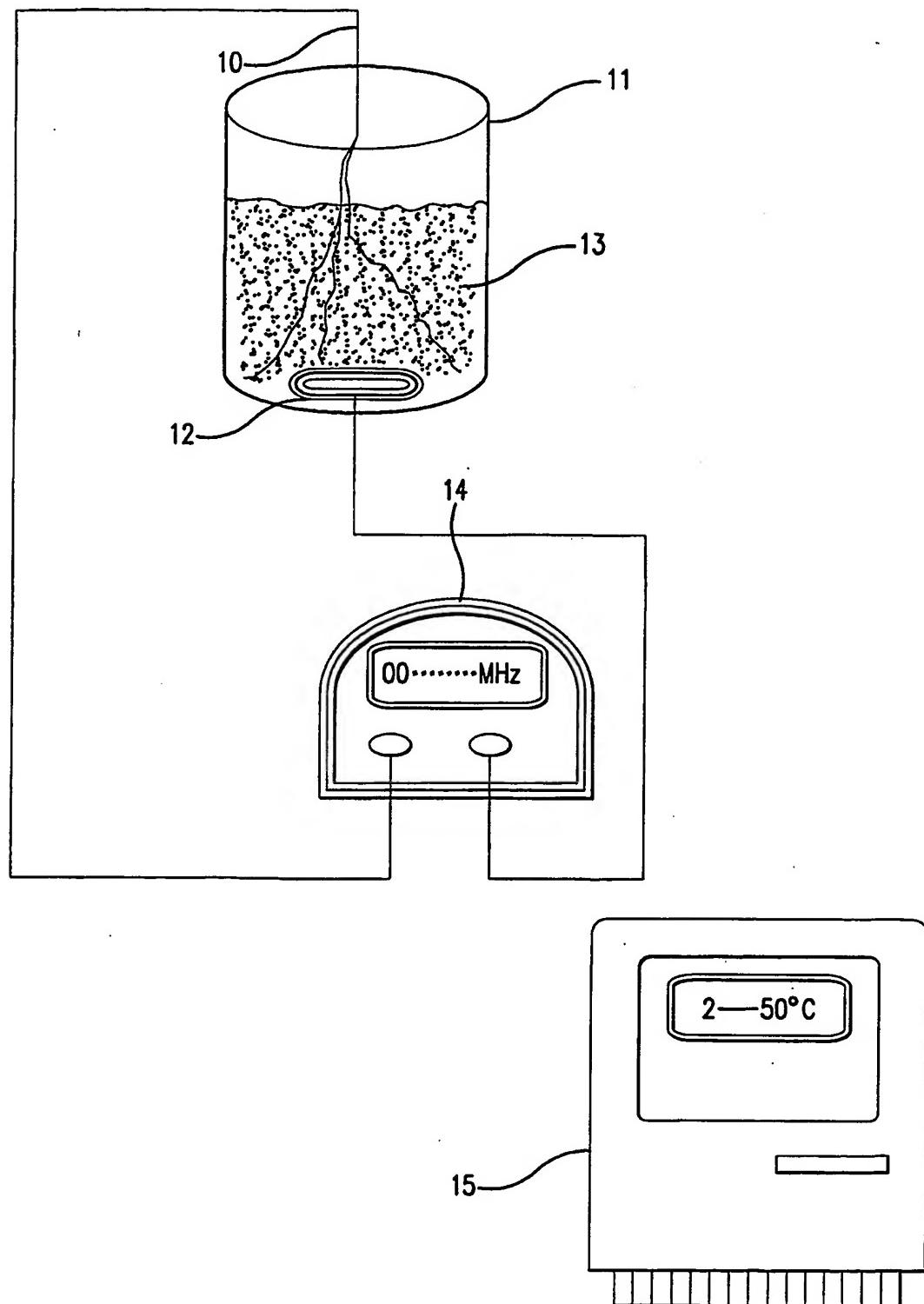


FIG.3

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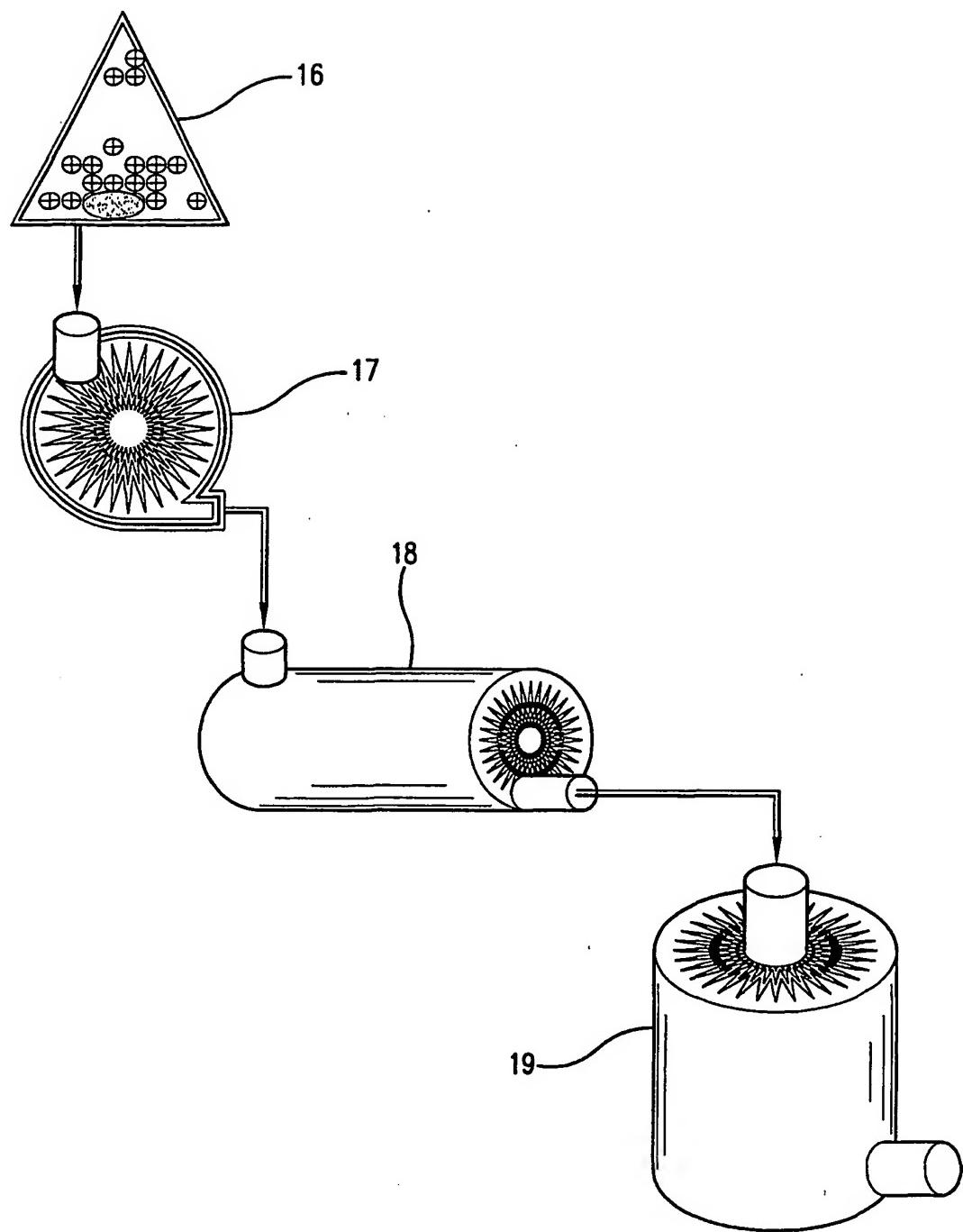


FIG.4

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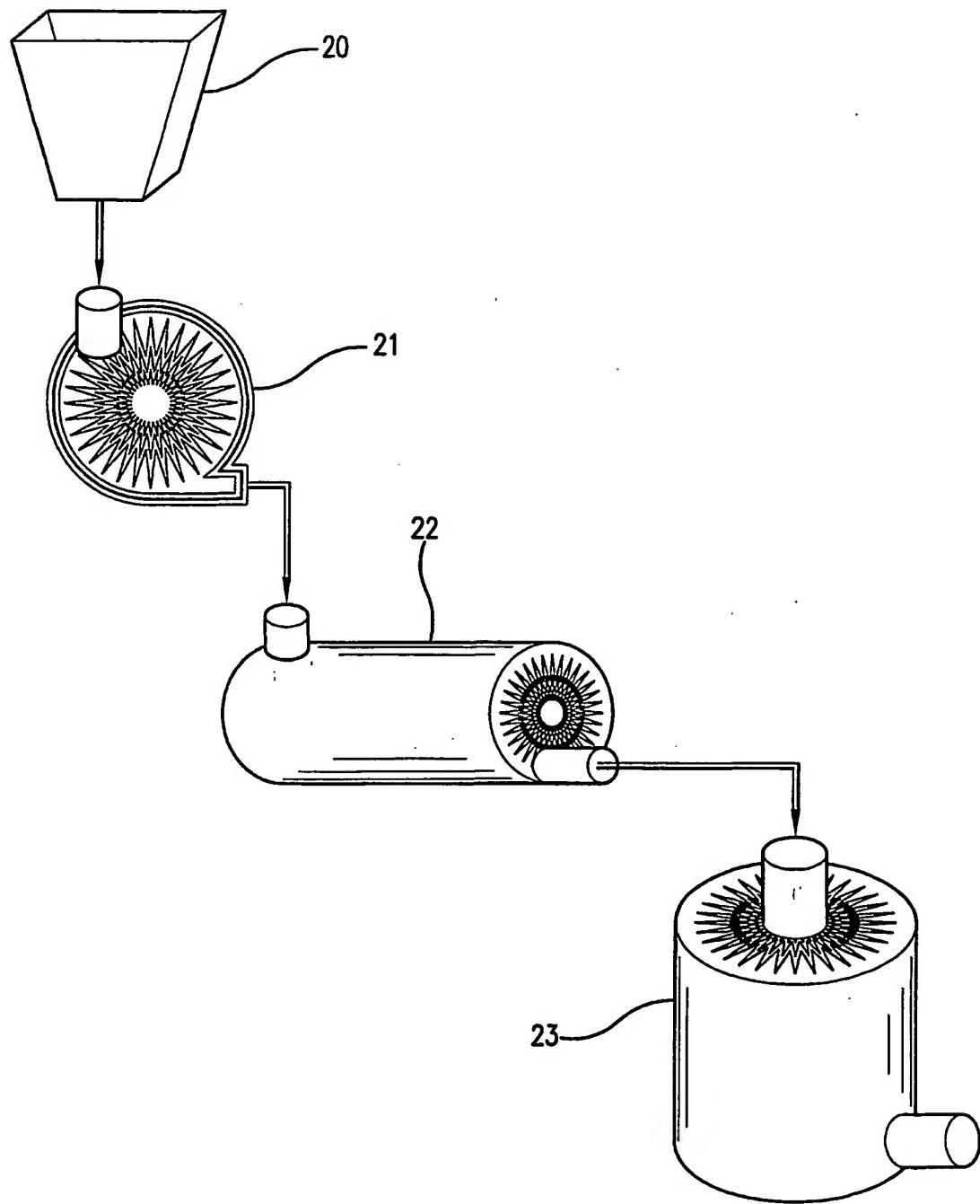


FIG.5

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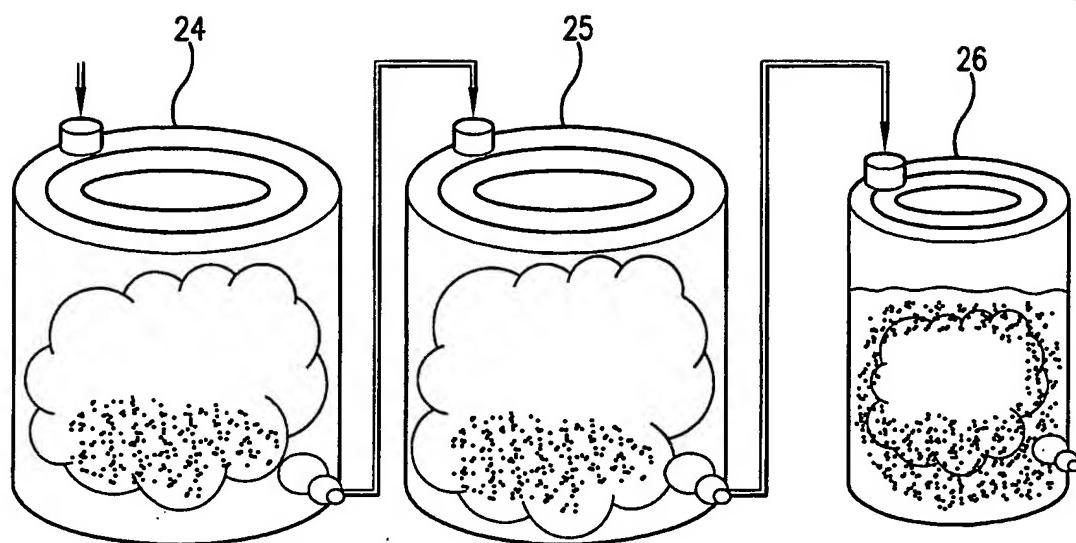


FIG.6

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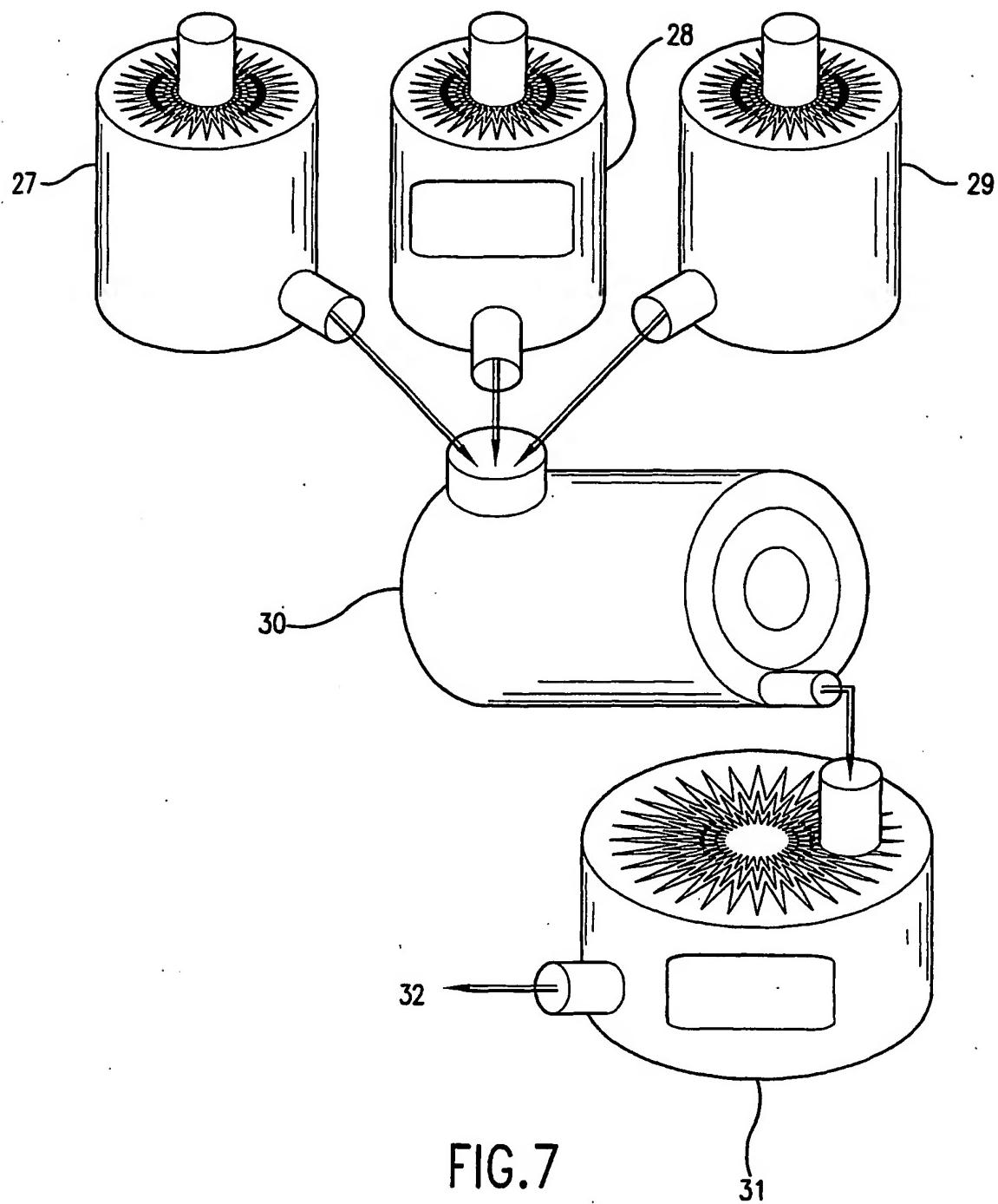


FIG.7

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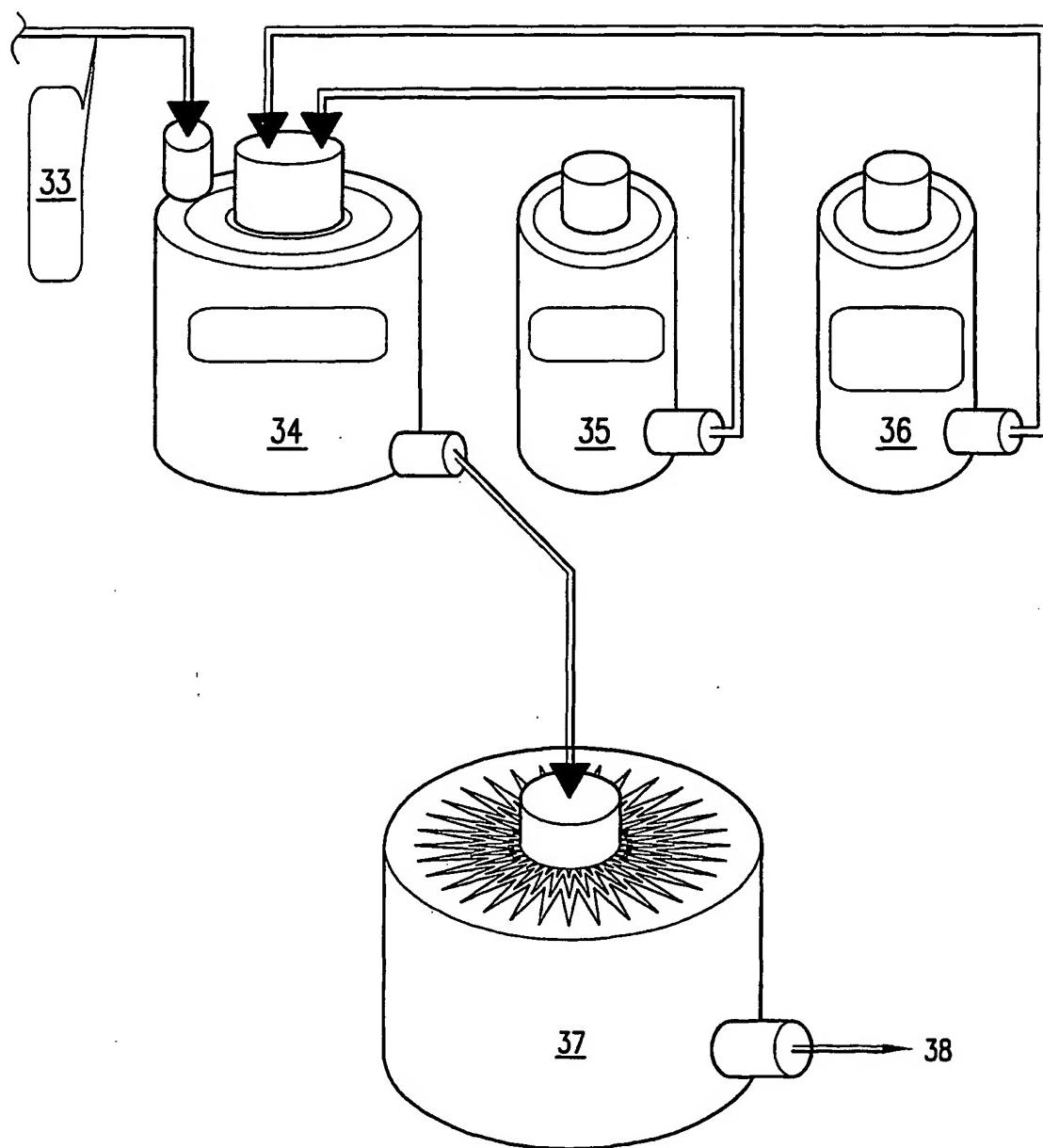


FIG.8

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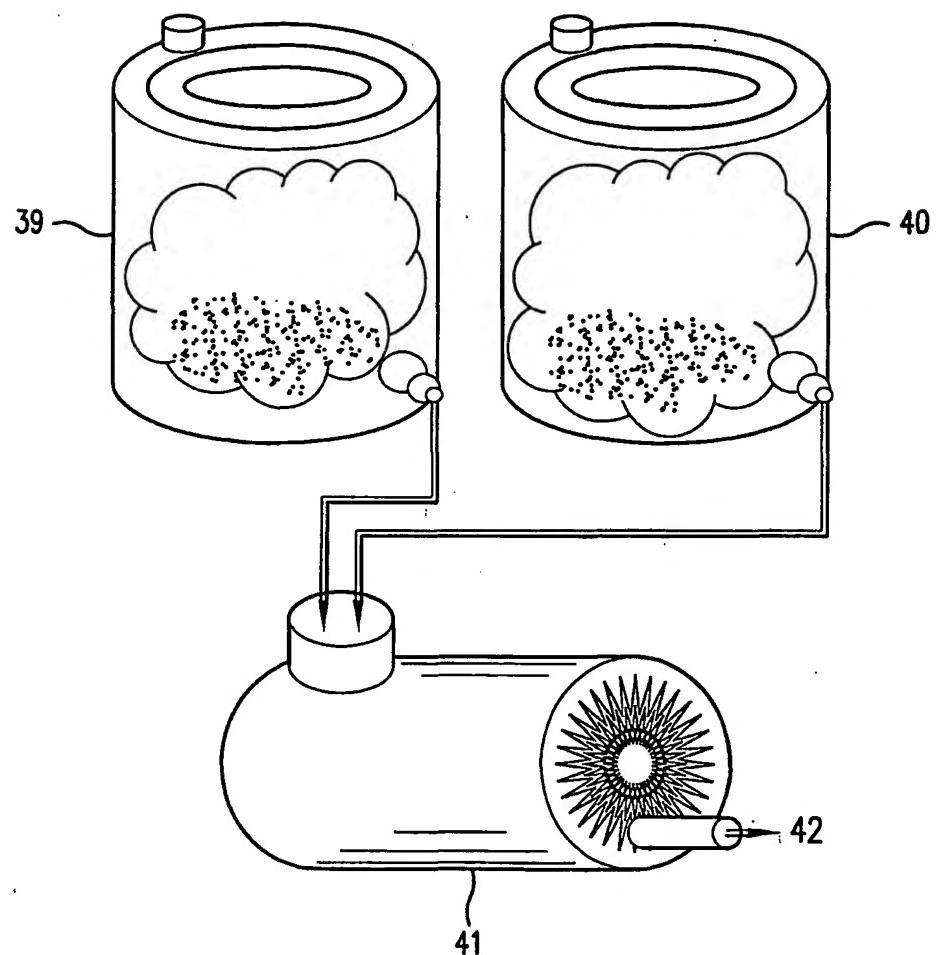


FIG.9

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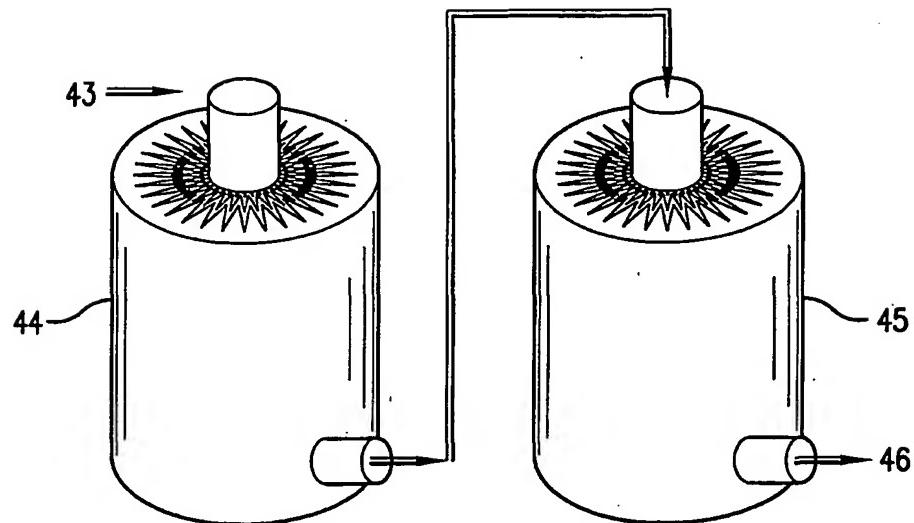


FIG.10

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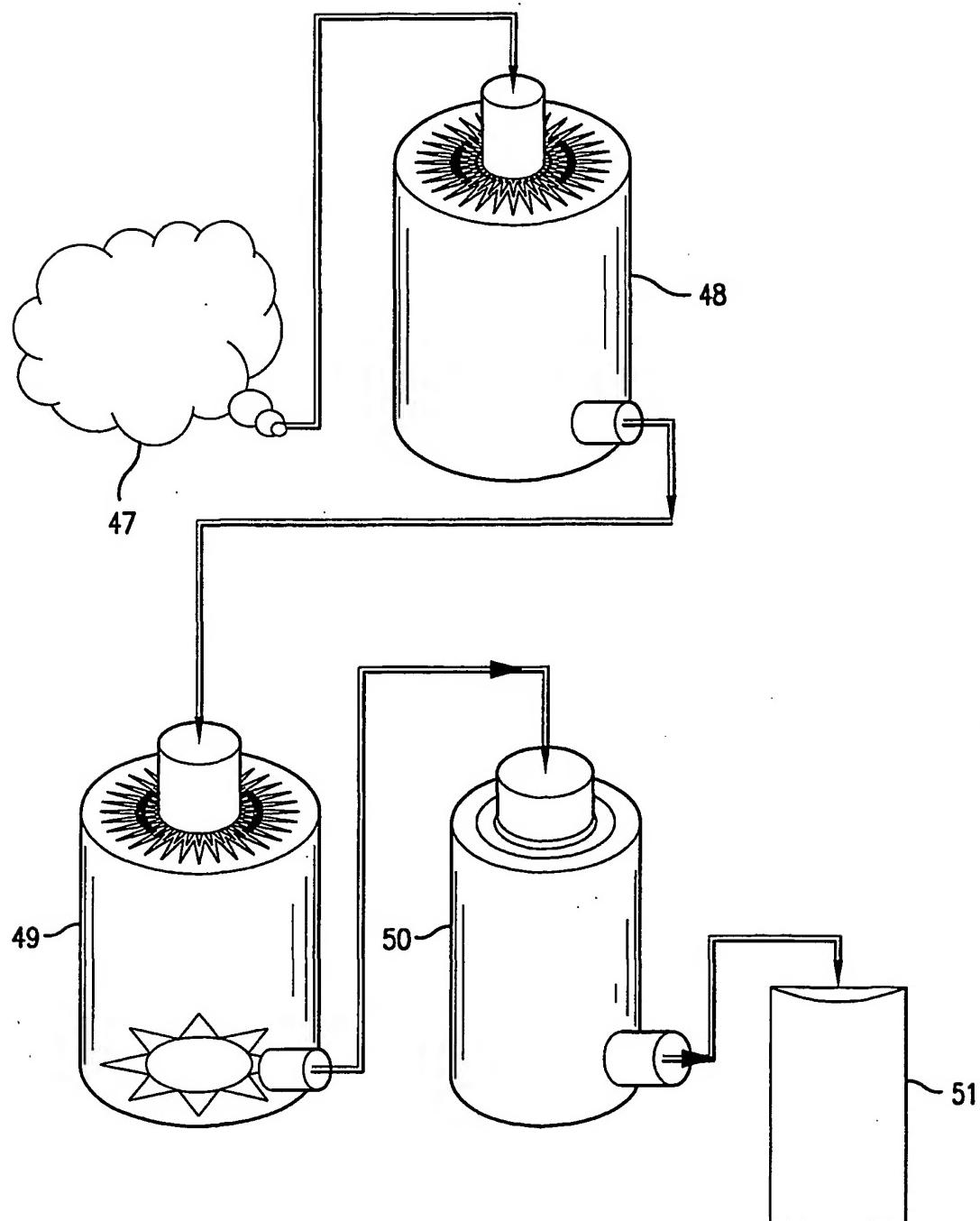


FIG.11

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
12 September 2002 (12.09.2002)

PCT

(10) International Publication Number  
**WO 02/070436 A3**

(51) International Patent Classification<sup>7</sup>: C05F 11/08, C12N 13/00, 1/16, 1/18

(21) International Application Number: PCT/GB02/00906

(22) International Filing Date: 1 March 2002 (01.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/796,818	1 March 2001 (01.03.2001)	US
09/796,819	1 March 2001 (01.03.2001)	US
09/796,820	1 March 2001 (01.03.2001)	US
09/796,821	1 March 2001 (01.03.2001)	US
09/796,822	1 March 2001 (01.03.2001)	US

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(74) Agents: MCCALLUM, William, Potter et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AB (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:  
17 July 2003

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A3

(54) Title: BIOLOGICAL FERTILIZER COMPOSITIONS COMPRISING MANURE, SLUDGE OR GARBAGE

(57) Abstract: The present invention provides biological fertilizer compositions that comprise yeast cells and an organic substrate. The yeast cells of the invention have an enhanced ability to fix atmospheric nitrogen, decompose phosphorus minerals and compounds, decompose potassium minerals and compounds, decomposes complex carbon compounds, overproduce growth factors, overproduce ATP, decompose undesirable chemicals, suppress growth of pathogenic microorganisms, or reduces undesirable odor. The organic substrates used can be manure, sludge, or garbage. The biological fertilizer composition of the invention can replace mineral fertilizers in supplying nitrogen, phosphorus, and potassium to crop plants. Methods of manufacture biological fertilizer compositions, and methods of uses are also encompassed.

**WO 02/070436 A3**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00906

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C05F11/08 C12N13/00 C12N1/16 C12N1/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 7 C05F C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, COMPENDEX, BIOSIS, INSPEC

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 02 20431 A (ULTRA BIOTECH LTD) 14 March 2002 (2002-03-14)  the whole document ---	1,2, 5-11, 13-29, 31-40
X	US 5 981 219 A (WEBER ANDREAS ET AL) 9 November 1999 (1999-11-09) column 1, line 14 - line 15 column 1, line 29 - line 32 column 4, line 41 - column 5, line 7 column 8, line 6 - line 33 examples 3,4 --- -/-	1,2,5-7

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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Date of mailing of the International search report

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 02/00906

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Derwent Publications Ltd., London, GB; Class C00, AN 1967-07448H XP002165714 & SU 220 916 A (KAMSKII CELLULOSE-PAPER WORKS) abstract ---	1,2,5-7
A	US 4 985 060 A (HIGA TERUO) 15 January 1991 (1991-01-15)  claims column 6, line 57 - line 64 ---	1,2, 5-11, 13-29, 31-40
A	US 4 119 429 A (LOVNESS DONALD E) 10 October 1978 (1978-10-10) claims column 2, line 38 -column 3, line 14 ---	1,2,5-11
X	FR 2 489 363 A (PASTEUR INSTITUT) 5 March 1982 (1982-03-05) the whole document ---	1,2
X	WO 95 04814 A (INT TLB RES INST INC) 16 February 1995 (1995-02-16) claims ---	1,2
A	WO 87 02705 A (SWEENEY GEORGE WILLIAM JR) 7 May 1987 (1987-05-07) claims -----	3-40 1-40

**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/GB 02/00906**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

3, 4, 12, 30 (1, 2, 5-11, 13-15, 16-29, 31-40) (PART)

4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (1,2,5-11,13-15,16-29,31-40) (partially)

Fertilizer composition comprising (I) an organic substrate, (II) a yeast cell component that fixes nitrogen and (III) at least one of the following: (d)a yeast component that suppress the growth of pathogenic microorganisms, (e) a yeast component that degrade antibiotics, (f) a yeast component that reduce the odor of the biological fertilizer composition.

2. Claims: (1,2,5-11,13-15,16-29,31-40) (partially)

Fertilizer composition comprising (I) an organic substrate, (II) a yeast cell component that decompose phosphorus and (III) at least one of the following: (d)a yeast component that suppress the growth of pathogenic microorganisms, (e) a yeast component that degrade antibiotics, (f) a yeast component that reduce the odor of the biological fertilizer composition.

3. Claims: (1,2,5-11,13-15,16-29,31-40) (partially)

Fertilizer composition comprising (I) an organic substrate, (II) a yeast cell component that decompose potassium compounds and (III) at least one of the following: (d)a yeast component that suppress the growth of pathogenic microorganisms, (e) a yeast component that degrade antibiotics, (f) a yeast component that reduce the odor of the biological fertilizer composition.

4. Claims: (1,2,5-11,13-15,16-29,31-40) (partially)

Fertilizer composition comprising (I) an organic substrate, (II) a yeast cell component that fixes nitrogen, a yeast cell component that decompose phosphorus compounds and (III) at least one of the following: (d) a yeast component that suppress the growth of pathogenic microorganisms, (e) a yeast component that degrade antibiotics, (f) a yeast component that reduce the odor of the biological fertilizer

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

composition.

5. Claims: (1,2,5-11,13-15,16-29,31-40) (partially)

Fertilizer composition comprising (I) an organic substrate, (II) a yeast cell component that decompose phosphorus compounds, a yeast cell component that decompose potassium compounds and (III) at least one of the following: (d)a yeast component that suppress the growth of pathogenic microorganisms, (e) a yeast component that degrade antibiotics, (f) a yeast component that reduce the odor of the biological fertilizer composition.

6. Claims: (1,2,5-11,13-15,16-29,31-40) (partially)

Fertilizer composition comprising (I) an organic substrate, (II) a yeast cell component that fixs nitrogen, a yeast cell component that decompose potassium compounds and (III) at least one of the following: (d)a yeast component that suppress the growth of pathogenic microorganisms, (e) a yeast component that degrade antibiotics, (f) a yeast component that reduce the odor of the biological fertilizer composition.

7. Claims: (1,2,5-11,13-15,16-29,31-40) (partially)

Fertilizer composition comprising (I) an organic substrate, (II) a yeast cell component that fixs nitrogen, a yeast cell component that decompose phosphorus compounds, a yeast cell component that decompose potassium compounds and (III) at least one of the following: (d)a yeast component that suppress the growth of pathogenic microorganisms, (e) a yeast component that degrade antibiotics, (f) a yeast component that reduce the odor of the biological fertilizer composition.

8. Claims: 3,4,5-10,12,13,15-28,30-40

Fertilizer composition as disclosed in claim 3 of the present application

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Inte	inal Application No
PCT/GB 02/00906	

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